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EFFICIENCY IN FIELD TRIALS OF PSEUDO-FACTORIAL AND INCOMPLETE RANDOMIZED BLOCK METHODS[1]

BY C. H. GOULDEN[2]

Abstract

Uniformity data for eight different crops were studied with the object of making comparisons of the efficiency of Incomplete and Randomized Block methods. Altogether 26 different comparisons were made.

In general the Incomplete Block method gives increases in efficiency, such increases being partially correlated with soil heterogeneity. If the field is very uniform there may be a loss in efficiency but this is rather unlikely on the average field and with careful planning of the experiment. The increases in efficiency due to the use of Incomplete Block methods would appear to vary on the average from 20 to 50%. In view of the greater adaptability of these methods to irregularly shaped fields, in addition to greater efficiency, their use can be generally recommended.

The relative efficiency of Incomplete and Complete Block methods was studied in relation to the size and shape of plots and blocks. The former method gives the greatest gains in efficiency when the Incomplete Blocks are nearly square and are made up of long narrow plots.

Introduction

For field tests involving a large number of varieties Yates (7, 8) has proposed the Pseudo-factorial and Incomplete Randomized Block methods. The Pseudo-factorial method in particular is recommended owing to its greater adaptibility with respect to the number of replications. From a study of uniformity data Yates (7) indicates that, if there is a sufficient degree of heterogeneity in the field, gains in efficiency by using the Pseudo-factorial method as compared to ordinary Randomized Blocks may range from about 20 to 50%. In the present paper results are given of studies with uniformity data from field experiments on the relative efficiency of Pseudo-factorial and Incomplete Blocks, with special reference to the degree of soil heterogeneity and to the size and shape of the plots and blocks.

It is a well known fact that compact plots or blocks of land are more variable than long narrow strips, although in the case of long narrow strips this only applies to strips that are placed side by side in the field. Therefore we must arrange field experiments so that the units designed for error control are as compact as possible and the units designed for comparing varieties or treatments within the error control units are as long and narrow as possible. Thus in a Randomized Block experiment the ideal arrangement is to have square blocks and each block divided into the required number of strips to be used

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as plots. Owing to practical considerations that limit plot shape this is not always possible, but we must always keep this principle in mind in designing an experiment, so as to approach as nearly as we can to the ideal situation.

In general, plot shapes vary from 1 : 5 to 1 : 20, and consequently groups of plots varying from 5 to 20 can be placed in square blocks. Therefore in designing an Incomplete Block experiment (for convenience including in this terminology both types mentioned above) it is generally much easier to make compact blocks than it is in the case of Randomized Blocks. Now in a study of uniformity data, in order to make a comparison of Incomplete and Randomized Block experiments, it is necessary to make both kinds of blocks as compact as possible to be fair to both arrangements; but to a certain extent this is unfair to the Incomplete Block design which has great adaptability to fields of various shapes and may give error control on fields where Randomized Blocks would be relatively inefficient. This is particularly true of fields that are very irregular or long and narrow. Furthermore in considering any particular set of uniformity data it may be obvious that a given type of Incomplete Block experiment will give the best results, but if this type cannot be compared with a Randomized Block experiment on the same field, then another type of Incomplete Block experiment, which is probably less efficient, must be used.

The procedure for making comparisons of the efficiency of the two methods is as illustrated in Fig. 1 and Table I. Fig. 1 is an outline to scale of a field of orange trees, the data from which were published by Batchelor and Reed (1). In this case each tree is taken as a single plot, and since the trees were the same distance apart in the rows and columns, the plots may be taken to be square. The portion of the field taken contains 810 trees and we can assume that it

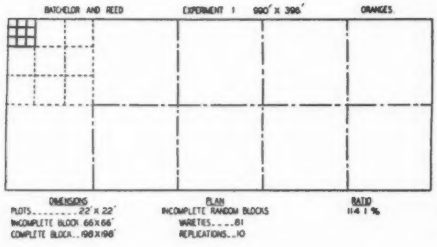


FIG. 1.

TABLE I*

ILLUSTRATING METHOD OF MAKING TESTS OF THE EFFICIENCY OF INCOMPLETE BLOCKS AS COMPARED TO RANDOMIZED BLOCK EXPERIMENTS

		SS	DF	MS
Incomplete Blocks	Blocks	1,004,655	89	11288
	Error	857,682	720	11912
	Total	1,862,337	809	
Complete Blocks	Blocks	654,078	9	72675
	Error	1,208,259	800	15103
	Total	1,862,337	809	

$$\text{Ratio} = \frac{15103}{11912} \times \frac{9}{10} = 114.1\%.$$

Data correspond to Fig. 1.

is being used to test 81 varieties in 10 replications. In the figure the dotted lines represent the Randomized Blocks that might be used. They are designated here as Complete Blocks in contra-distinction to Incomplete Blocks. One Complete Block is shown divided into Incomplete Blocks and one of the latter is shown divided into plots. The dimensions of all of the units can then be seen in relation to each other. Here we have square plots and square blocks of both kinds. The arrangement of the square plots in square blocks is obviously the most desirable from the standpoint of error control. The test of efficiency is made by first carrying out a simple analysis of variance for each design as in Table I. We note that the Incomplete Blocks result in a lower error variance; but this is not pure gain in efficiency, as in comparing two varieties by this method the variance of a mean difference as ordinarily computed must be multiplied by an efficiency factor arising from the particular type of Incomplete Block experiment used. Here the efficiency factor, which is explained in more detail below, is 9/10. Where the error mean square arising from the Incomplete Block experiment is error (*i*) and that from the Complete Block experiment is error (*c*) the relative efficiency in percentage of the former is given by the ratio—

$$\frac{\text{Error } (c)}{\text{Error } (i)} \times \text{Efficiency Factor} \times 100.$$

Thus if our ratio is 120%, the gain in efficiency due to using Incomplete Blocks is 20%.

The Efficiency Factor

The efficiency factor of Incomplete Block experiments must be considered in some detail in making efficiency tests, as in certain cases an Incomplete Block experiment may be considered as belonging to one of several types, and one has to decide whether it is justifiable to use the type having the highest efficiency factor. Thus a uniformity experiment assuming 64 varieties to be tested in Incomplete Blocks of eight plots, and with six replications, may be considered as a simple Two Dimensional Pseudo-factorial with two groups of sets, or as a Two Dimensional Pseudo-factorial with six groups of sets, one for each replication. In the first case the calculation of the corrected variety means and the variety sum of squares is relatively simple but in the second case the calculations are somewhat involved. The method of carrying out the calculations in the second case has apparently not yet been published but it is merely an extension of the method given by Yates (7) for Two Dimensional Pseudo-factorials with three groups of sets. In the first case the efficiency factor is $9/11 = 0.8182$, and in the second case it is $45/51 = 0.8824$. In considering exactly the same uniformity data, therefore, the greatest efficiency can be obtained by assuming the experiment to be one with six groups of sets. This method would probably not be used in actual practice, but it would seem to be justifiable to consider the theoretical experiment as such owing to the fact that the reason we have only six replications is that the data are limited. In designing an actual experiment we would probably endeavor to use nine replications, in which case we would have an Incomplete Block

experiment of the symmetrical type for which the calculations are quite simple, and for which the efficiency factor has a maximum value of 9/10.

The efficiency factor for any Incomplete Block experiment of the Two Dimensional type is given by—

$$E = \frac{(k-1)(p+1)}{(k-1)(p+1)+k}$$

where k represents the number of groups of sets and p is the number of plots in an Incomplete Block. This formula does not hold for the symmetrical type of experiment for which the efficiency factor is always $\frac{p}{p+1}$.

The changes in the efficiency factors for different values of k and p are illustrated in Fig. 16 for $k = 2$ to 11 and $p = 4$ to 12. An important feature of this graph is that for the higher values of p the increase in the efficiency factor is very small after we pass $k = 6$ or 7. However even for $k = 5$ the computations in actual practice are quite cumbersome, so that the experimenter will rarely ever go beyond $k = 4$ unless he can go to the limit, whereupon the computations very suddenly become simplified. Of course in certain cases, such as in a test of 36 varieties, it is impossible to go beyond $k = 3$ and therefore the Pseudo-factorial method is the only one available. In general the difference between $k = 2$ and $k = 3$ is quite marked and consequently it would seem advisable where reasonably good computational facilities are available to use the three groups of sets.

Results of Efficiency Tests

The results of the efficiency tests are presented in Figs. 1 to 15, which are all of a type similar to Fig. 1 which has been described. Since the results of such tests are only of interest in relation to the particular layout considered, this method of presentation has been followed throughout. The method of investigation was to take a set of uniformity data and arrange comparisons of Incomplete and Randomized Block experiments in different ways. For example the data published by Sayer (4) consists of yields of sugar cane plots measuring 3 × 60 ft. As illustrated in Fig. 5, the first experiment consisted of Incomplete Blocks measuring 24 × 60 ft. and Complete Blocks measuring 120 × 196 ft. The latter were therefore more compact than the former, and as a result, the gain in efficiency due to the use of Incomplete Blocks is only 7.3%. In Experiment II the Incomplete Block is the most compact and the effect of this is shown in the efficiency gain of 15%. Finally as in Experiment III the Complete Blocks are again the most compact but the original plots have been combined in fours and a great deal of the irregular soil variability within Incomplete Blocks has been removed. The result is a gain in efficiency of 37%. The combining of plots in order to remove minor soil variations from within the blocks seems in general to be profitable, and would appear to be due to the smoothing out of variations due to direct accidents to individual plots such as weed growth, cracking of the soil, variations in stand, and so forth. These are rather different from other factors

affecting soil variability, such as soil texture, moisture supply, and general fertility. When the minor factors are largely overcome, the plot yields tend to reflect true patchiness effects and fertility trends. It is these variability factors that are removed by the Incomplete Block method and, consequently, the best results are obtained when the minor variability factors are largely eliminated.

The series of orange-tree yields given by Batchelor and Reed are of especial interest in relation to the efficiency of the Incomplete Block method. The plots are single-tree yields, each tree occupying an area 22 feet square. In Fig. 1, the original plots are shown combined in square Incomplete and square Complete Blocks. The efficiency test is therefore perfectly balanced with respect to the two methods. The gain due to the Incomplete Blocks is 14.1% and the reason that this is not a large gain is probably the fact that square plots, although arranged as compactly as possible in the blocks, are not particularly efficient. In Fig. 3 the plots are shown combined in twos, and while the Incomplete Blocks are not as compact as the Complete Blocks, the gain in efficiency is now 25.5%. In Fig. 4 the plots have been combined in fives and now represent reasonably efficient units. The Incomplete Blocks are still not as compact as the Complete Blocks but the gain is now 47.7%.

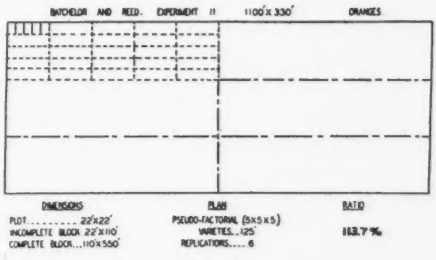


FIG. 2.

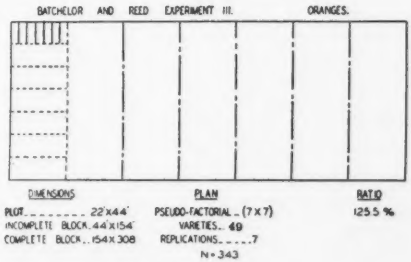
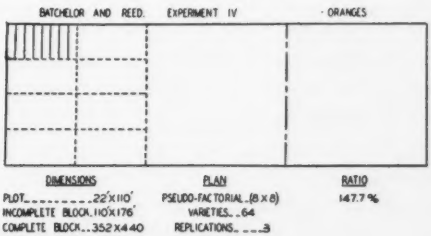


FIG. 3.



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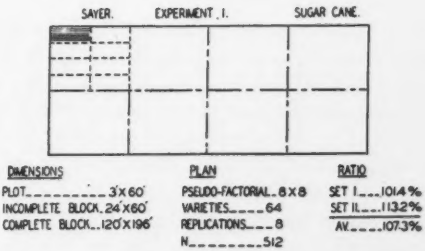
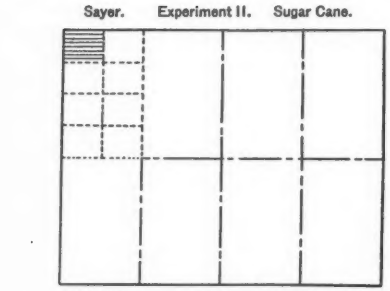


FIG. 5.



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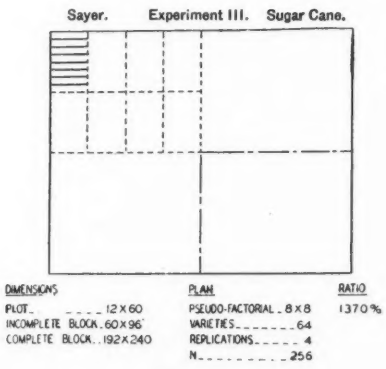

FIG. 7.

It should be made clear at this point that the change in plot shape is considered only in relation to the relative efficiency of Complete and Incomplete Block experiments. For example in the case cited above it is not argued that the plots combined in fives are more efficient than if in the same area the original square plots are used with five times as many replications. In actual practice the experimenter will have to balance up the two factors, namely, the increase in efficiency due to making the Incomplete Blocks compact and the increases in efficiency due to greater replication with less efficient blocks. For plots of equal area, however, the above results apply directly, since the number of replications will remain the same; and in general the assumption may be made, for example, that if the square plots in Batchelor and Reed Experiment I were of the same area as those in Experiment IV the increase in efficiency would not be as great as in the latter case.

Fig. 2 is an illustration, with the Batchelor and Reed data, of a Three Dimensional Pseudo-factorial experiment. The varieties are assumed to number 125 and these are tested in blocks of five plots using three groups of sets. The gain in efficiency of 13.7% is quite satisfactory, considering that the Incomplete Blocks are not very compact and the original single plot yields are used. If sufficient plots were available to allow for combining them in fours, a much greater gain in efficiency would be expected.

The experiments with the square yard barley yields illustrated in Figs. 8 and 9 form a series very similar to those for the Batchelor and Reed plots except that only two good arrangements were possible. The square plots do not give any increase in efficiency, in fact there is a loss of 7.1%. When combined in eights to form plots 1 yd. × 8 yd. the increase in efficiency is 17.9%, and this may be considered reasonably satisfactory. Fig. 10 illustrates an experiment in which a deliberate attempt was made to obtain a reduction in efficiency by making the Incomplete Blocks long and narrow and the Complete Blocks compact. The result was a reduction in efficiency of 37.2%,

and illustrates very clearly that success with the use of Incomplete Blocks does not arise from the method in itself but from the fact that it allows for the planning of an experiment in such a way as to give real increases in efficiency.

The results with potato yields from Kirk and Goulden (3) is an example of a field which does not permit of a comparison using compact Complete Blocks. The yields given were actually for varieties in a Randomized Block experiment and therefore the yield of each plot was corrected for the variety effect. As would be expected, the results are erratic. Two sets of data were available, one set giving practically no increase in efficiency while the other set gave an increase of 183.1%. This result may be used as an illustration of the adaptability of the Incomplete Block method to fields of varying shape. To use Complete Blocks of the type illustrated in Fig. 11, on account of irregularities or roadways in the field, would obviously give on the whole very bad results. However, on any such field the Incomplete Blocks would allow the setting up of an efficient design.

The results for the data by Summerby (5) illustrate again the unpracticability of square plots, especially when they are combined in Incomplete Blocks that are long and narrow. Throughout the seven sets studied the plan was the same, the Incomplete Blocks being narrow and the Complete Blocks square. In view of this fact it is remarkable that the efficiency ratios average 100.8%. In spite of the poor layout there was on the average no loss in efficiency.

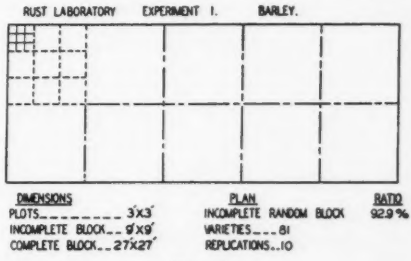

FIG. 8.

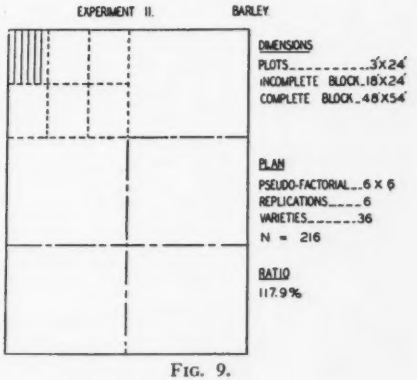

FIG. 9.

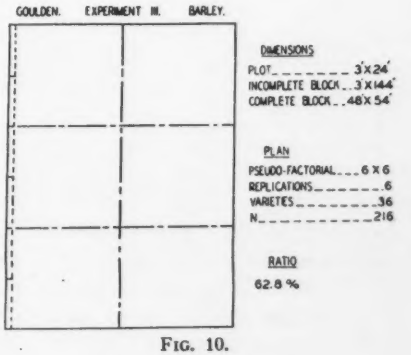

FIG. 10.

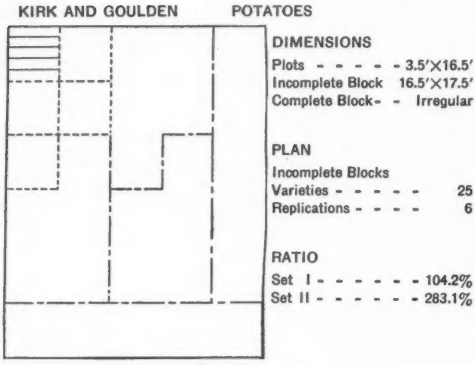

FIG. 11.

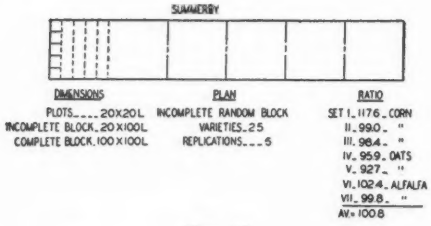

FIG. 12.

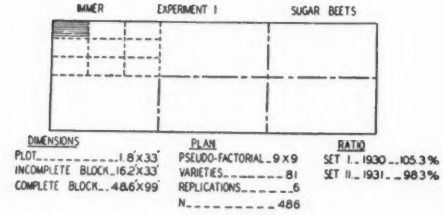

FIG. 13.

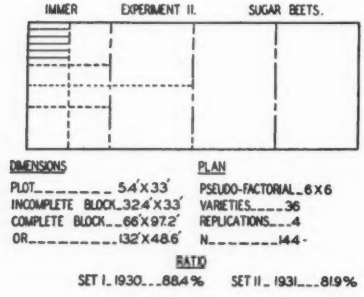

FIG. 14.

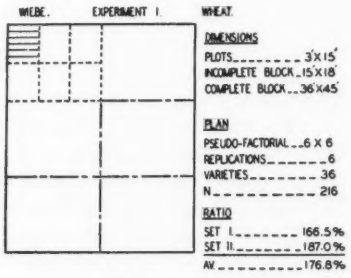

FIG. 15.

The sugar beet plots came from one set unpublished but furnished by the courtesy of Dr. F. R. Immer and another set from a similar nearby field on which certain results have already been given by Immer (2). These plots are of interest in that, in spite of a favorable plan for the assumed Incomplete Block experiment in both Experiments I and II, the first gave an average ratio, for the two sets, of only 101.8%, and the second of only 85.2%. This field appears to be exceptionally uniform, and in such cases it does appear that there is a decided possibility of a loss in efficiency through using the Incomplete Block method. It would be of interest here, however, to study in further detail the causes of plot variability. If variability from plot to plot is caused by variations in stand and other factors of that nature it might easily be that the Incomplete Block method would be incapable of bringing about any improvement in efficiency.

The results for the data by Wiebe (6) are in direct contrast to those for the data by Immer. In the former, the plots show a decided variability and this is reflected largely in patchy effects and fertility gradients. The plots were first combined in threes but still provided a sufficient number of plots to give two sets of data for which efficiency ratios could be calculated as indicated in Fig. 15. The average efficiency ratio was 176.8%. This result

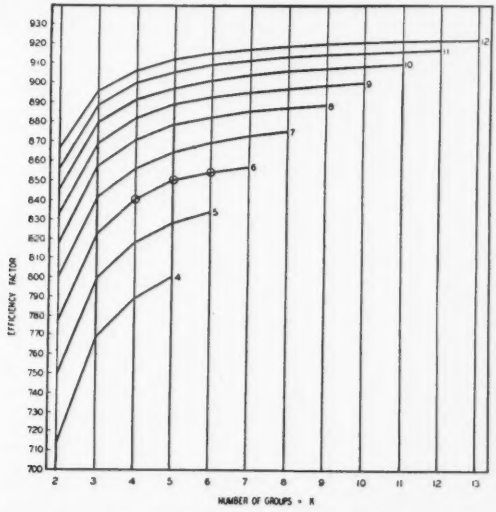

FIG. 16. *The changes in the efficiency factor for $k = 2$ to 11, and $p = 4$ to 12. The points covered by small circles are impossible owing to the impossibility of forming a completely orthogonalized (6×6) square.*

is particularly important in that a great many yield trials of wheat varieties are conducted in plots of approximately the same shape as in this study. The increase in efficiency of about 77%, if general throughout such trials, would be extremely important.

From the data studied it is very difficult to come to any general conclusion as to the increase in efficiency to be expected from the use of the Incomplete Block methods. Under average conditions and with careful planning of the experiment, increases in efficiency ranging from 20 to 50% would appear to be possible. In certain cases, with very uniform fields, there may be a slight loss in efficiency, but such fields are probably rare. With very patchy fields on which experimenters frequently have to work, the gain in efficiency may easily exceed 50% and may be as great as 100%.

The reason for the increased efficiency of Incomplete Block as compared to Randomized Block field trials is to be found obviously in the relative efficiency for error control being sufficiently great to more than overcome the efficiency factor. The point of relative efficiency in error control of the

two types of blocks is rather important, as it might be assumed that the Incomplete Blocks would always give higher efficiency with increasing heterogeneity. That this is not the case is obvious from Table II which gives for each of the cases investigated the efficiency ratio, the intra-class correlation (r_i) for the Incomplete Blocks and the intra-class correlation (r_c) for the

TABLE II

EFFICIENCY RATIOS FOR ALL SETS OF DATA STUDIED AND THE CORRESPONDING INTRA-CLASS CORRELATION COEFFICIENTS FOR THE INCOMPLETE AND COMPLETE BLOCKS

——	Exp.	Set	Ratio	r_i	r_c	$r_i - r_c^2$
Rust Laboratory	III		62.8	0.2666	0.4470	0.067
Immer	II	2	81.9	.0472	−.0066	.047
Immer	II	1	88.4	.1382	.0210	.138
Summerby	I	5	92.7	.5546	.5409	.262
Rust Laboratory	I		92.9	.0738	.0475	.072
Summerby	I	4	95.9	.2479	.1498	.225
Immer	I	2	98.3	.0930	−.0005	.093
Summerby	I	3	98.4	.3883	.3058	.295
Summerby	I	2	99.0	.3782	.2886	.295
Summerby	I	7	99.8	.7324	.7103	.228
Kirk	I	1	101.4	.6127	.5646	.294
Sayer	I	1	101.4	.0180	.0687	.013
Summerby	I	6	102.4	.7027	.6678	.257
Immer	I	1	105.3	.2038	.0386	.202
Sayer	I	2	113.2	.4255	.2892	.342
Batchelor, Reed	II		113.7	.5645	.3513	.441
Batchelor, Reed	I		114.1	.4850	.3678	.350
Sayer	II		115.0	.3801	.2138	.334
Summerby	I	1	117.6	.4932	.3120	.396
Rust Laboratory	II		117.9	.6122	.4470	.412
Batchelor, Reed	III		125.5	.6307	.5006	.380
Sayer	III		137.0	.4575	.2380	.401
Batchelor, Reed	IV		147.7	.6789	.5323	.396
Wiebe	I	1	166.5	.7529	.5058	.497
Wiebe	I	2	187.0	.7151	.3420	.598
Kirk	I	2	275.3	.8377	.4953	.592

Complete Blocks. Since the Incomplete Blocks are relatively small units, the corresponding intra-class correlation may be taken as a general measure of soil heterogeneity. There is a certain degree of correlation between this measure and the efficiency ratio in that we must have fairly high values of r_i before high efficiency ratios can be obtained, but in certain cases of high values of r_i the efficiency ratio is low. This arises from the fact that in these cases r_c is also high. As examples of this fact we may take the two cases as given in Table II for Summerby Experiment I, Set 7, and Sayer Experiment III. In the first we have $r_i = 0.7324$, and $r_c = 0.7103$. In the second we have $r_i = 0.4575$ and $r_c = 0.2380$. An arbitrary constant which seems from a preliminary examination of the data to be reasonably well correlated with the efficiency ratio is $(r_i - r_c^2)$. This is given in the last column of Table II.

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LABORATORY MALTING. II. PRECISION[1]

By J. Ansel Anderson[2] and W. O. S. Meredith[3]

Abstract

The precision of the malting test made in equipment already described (Can. J. Research, C, 15 : 204-216. 1937) was studied by making four batches of malt each of which contained duplicate malts made by eight treatments representing the combination of steeping for 48 or 60 hr., maintaining the germination chamber at 53° or 54.5° F., and kilning for 36 hr. at 100° to 175° F. or for 52 hr. at 90° to 165° F. The standard errors of duplicate tests made in the same and in different batches were found to be: extract, 0.08 and 0.09%; moisture, 0.04 and 0.05%; color, 0.04 and 0.05 units; diastatic power 1.0 and 2.8° L.; permanently soluble nitrogen as percentage of wort solids, 0.01 and 0.02%; malting loss 0.06 and 0.29%; and sprouts, 0.06 and 0.18%.

On the average, increasing the time of steeping decreased extract by 0.08%; but increased diastatic power by 3.2° L., permanently soluble nitrogen by 0.05%, malting loss by 0.98% and sprouts by 0.44%. Increasing the temperature of the germination chamber increased diastatic power by 4.2° L., permanently soluble nitrogen by 0.04%, malting loss by 0.93%, and sprouts by 0.52%. Increasing the time and decreasing the temperature of kilning increased extract by 0.08% and diastatic power by 14.8° L. Statistical analyses show that the test is sufficiently precise to prove that these effects, though small, are significant.

It is apparent that the usefulness of a laboratory malting test as a research tool will be limited by the precision of the test. The greater the precision, *i.e.*, the more nearly it is possible to reproduce identical malts from the same barley, the less will be the replication required to prove whether significant differences exist between samples of very similar malting qualities, the finer will be the differentiation between samples, and the greater will be the ease with which the effects of variety and environmental conditions on malting quality can be demonstrated. For these reasons, and because comparisons based on methods of unknown precision lose much of their value, it would appear that it is the first duty of any new malting laboratory to determine the level of precision of its malting test.

The investigation reported in this paper was undertaken primarily with the object of determining the level of precision of the malting test developed in the National Research Laboratories during the past year and the scope of the work which could be undertaken with it. It also serves to demonstrate the design of investigation for which the malting equipment is particularly suited, namely, a factorial design involving the simultaneous study of three factors, each of which is tested at two levels.

Design of Investigation

The investigation was made with equipment which consists of duplicate sets of steep tank, germination chamber and kiln, each set having a capacity of eight 350-gm. samples (2). Four batches of 16 malts were made under as

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nearly identical conditions as the precision of control in the equipment would permit. In each batch, eight samples were steeped in each tank, and four samples from each tank were then put into each germination chamber. At the end of the germination period there were thus four sets of quadruplicate samples, each set having been exposed to a different combination

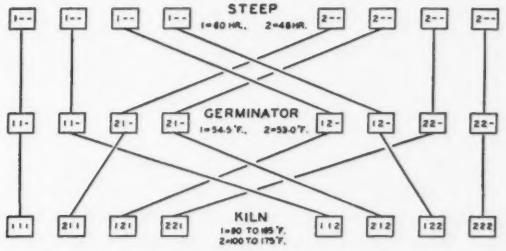

FIGURE I. *Factorial distribution of samples. First digit = steep; second digit = germination; third digit = kiln.*

of steeping and germination conditions. Two samples from each of these four sets were then placed in each kiln; thus after kilning, there were eight sets of duplicate samples, each of which had been exposed to a different combination of malting conditions. The eight treatments will hereafter be designated by numbers of three digits, the first representing the steeping procedure, the second the germination procedure and the third the kilning procedure (Fig. 1).

Methods

Steeping

In Procedure 1 the samples were steeped for 60 hr. at 50° F., in Procedure 2 the time was reduced to 48 hr.

The samples were steeped for the first 48 or 36 hr. in quart sealers, and for the last 12 hr. they were steeped in 8-mesh cages. During the first period, the water was decanted and the samples were allowed to remain without water for 1 hr. at the end of each 12 hr.

Germination

In Procedure 1 the chamber was maintained at 54.5° F. and in Procedure 2 the chamber was maintained at 53.0° F.

After being in the chamber for 8 hr. in 8-mesh cages the samples were transferred to galvanized iron cylindrical cans with eight $\frac{1}{8}$-in. holes. At 120 hr. the samples were transferred back to 8-mesh cages to permit some withering.

All samples were rotated continuously at 0.05 r.p.m. and were treated with 15 ml. of water at 72 hr.

Kilning

Procedure 1 consisted of kilning for 52 hr. at temperatures of 90° to 165° F. and Procedure 2 of kilning for 36 hr. at temperatures of 100° to 175° F.

The details were as follows:— No. 1, 8 hr. at 90° F., 18 hr. at 120° F., 22 hr. at 135° F., and 4 hr. at 165° F.; No. 2, 8 hr. at 100° F., 18 hr. at 130° F., 6 hr. at 145° F., and 4 hr. at 175° F. The samples were held in 8-mesh cages and were rotated continuously at 0.8 r.p.m.

General

In order to make sure that differences between the malts were the result of the controlled differences in malting procedure, and not of uncontrolled idiosyncrasies of any unit of the equipment, the procedures were used alternately in each set of equipment.

Methods for Malting Loss and Sprouts

Immediately after kilning the malt was kneaded in a small bag. The sprouts which were rubbed off during this process were separated by sifting and weighed to the nearest 0.1 gm. The polished malt was weighed to the nearest 0.5 gm. Malting loss and sprouts are reported as grams per 100 gm. of barley dry matter.

Analytical Methods

Each malt was divided into duplicate samples with a Boerner sampler. The samples were then analyzed in random order for extract, moisture, color, diastatic power and permanently soluble nitrogen.

Extract (fine grind) and moisture were determined by the Official Methods of the American Society of Brewing Chemists (1). Color was determined by the Official Method except that a Lovibond tintometer, made by the British Drug Houses, Limited, was used.

Diastatic power was determined by the modification of the Official Method proposed by Anderson and Sallans (3).

The permanently soluble nitrogen in the wort was determined by adding 10 ml. of a buffer solution, containing equal parts of normal acetic acid and normal sodium acetate solutions to 50 ml. of wort, heating for 30 min. in a boiling water bath, filtering, transferring 25 ml. of the filtrate to a Kjeldahl flask, evaporating to a thick syrup on the steam bath, and determining the nitrogen by the Kjeldahl method. Permanently soluble nitrogen is reported as a percentage of total wort solids.

Materials

A bulk lot of O.A.C. 21 barley, having a nitrogen content of 2.06% and a 1000-kernel weight of 32.1 gm., was split with a Boerner sampler into 64 samples each representing 300 gm. of barley dry matter. The samples were malted in random order.

Experimental Results

The experimental data are too numerous to permit publication of all of them, but excerpts from them and various summaries are presented in Tables I to V. A non-statistical discussion of results is presented in the next section, which is followed by a short section giving the results of the statistical analyses.

Discussion

Precision of Malting Tests made in the Same Batch

The precision of comparisons of samples that are malted in the same batch is affected by two errors, that of malting and that of analysis. Information on both errors is provided by the investigation as each batch contained eight sets of duplicate malts and each malt was subjected to duplicate analyses. The complete data for extract and diastatic power from Batch 1 are presented in Table I in the form of differences between duplicate malting tests and between duplicate analyses. It will be observed that the former are of little greater magnitude than the latter.

TABLE I

COMPLETE DATA FOR BATCH I FOR EXTRACT AND DIASTATIC POWER IN TERMS OF DIFFERENCES BETWEEN DUPLICATE MALTING TESTS AND BETWEEN DUPLICATE ANALYSES

Treatment	Extract, %		Diastatic power, °L.	
	Duplicate malts	Duplicate analyses	Duplicate malts	Duplicate analyses
111	.40	.12 .09	1.6	0.0 3.7
112	.30	.09 .05	1.7	0.1 0.2
121	.13	.22 .12	0.4	0.2 3.8
122	.06	.15 .03	0.8	0.8 0.8
211	.22	.13 .21	1.6	0.0 0.6
212	.06	.22 .11	0.3	3.3 3.4
221	.18	.11 .04	4.8	1.8 2.9
222	.16	.07 .23	0.6	1.3 0.3

A more concise picture of the errors can be obtained by presenting them in the form of standard errors. These have been calculated for all determinations from the complete data of the investigation and are reported in the first two columns of Table II. Since both malting tests and analyses will generally be made in duplicate, it seemed best to report the standard errors for means of duplicate tests and analyses.

The data show that the standard errors for tests made in the same batch are of about the same magnitude as those for analyses. It must be concluded, therefore, that the errors of malting are comparatively small and are masked by the errors of analysis, even though these are also small. For diastatic

TABLE II

STANDARD ERRORS FOR THE MEANS OF DUPLICATE ANALYSES AND DUPLICATE MALTING TESTS MADE IN THE SAME AND DIFFERENT BATCHES

Determination	Duplicate analyses	Duplicate malting tests	
		In same batch	In different batches
Extract, %	.07	.082	.093
Moisture, %	.038	.037	.045
Color, Lovibond units	.049	.043	.049
Diastatic power, °L.	.80	.99	2.77
Permanently soluble nitrogen as % of wort solids	.016	.011	.024
Malting loss, %	—	.065	.292
Sprouts, %	—	.058	.179

power and extract, the standard errors for tests are larger than those for analysis, but statistical analyses showed that the difference did not attain a 5% level of significance. In other words, the available data fail to prove that the variation between duplicate tests cannot be accounted for by chance combinations of variations in analytical results. It appears that the level of precision attained can be considered satisfactory and that if improvement is desired it will be necessary first to increase the precision of the analyses.

Precision of Malting Tests Made in Different Batches

In any but very small investigations it will be impossible to base all comparisons on tests made in the same batch. It is therefore necessary to consider the precision of tests made in different batches. This will be affected by an additional error which represents the variations in malting conditions between batches, which are the result either of personal errors or of failure of the equipment to maintain exactly the same set of conditions over extended periods.

The standard errors for the means of duplicate tests made in different batches are reported in the last column of Table II. Since only four batches of malt were made, the data must be considered only as a rough estimate of the errors. It is apparent that the variation between batches adds very considerably to the error of the test.

Further information on the variation between batches can be obtained from Table III. The data represent the means, over all treatments, for each determination and for each of the four batches. It is evident that the results for Batch 4 are considerably higher than those for the other three batches. The malting records show that the temperature controls for the steep tanks failed to function properly when Batch 4 was being made, owing, apparently, to a sudden increase in room temperature during a spell of mild weather. The temperature of the steeping water was more than 2° F. too high for part of the time.

TABLE III

MEANS, OVER ALL TREATMENTS, FOR EACH DETERMINATION AND EACH BATCH

Determination	Batch 1	Batch 2	Batch 3	Batch 4	Range
Extract, %	75.25	75.20	75.22	75.36	0.16
Moisture, %	3.63	3.68	3.62	3.69	0.07
Color, Lovibond units	2.13	2.17	2.12	2.20	0.08
Diastatic power, °L.	128.5	130.6	127.4	136.0	9.2
Permanently soluble nitrogen as % of wort solids	1.46	1.46	1.43	1.50	0.07
Malting loss, %	10.73	10.85	10.41	11.38	0.97
Sprouts, %	4.35	4.47	4.32	4.85	0.53

On the whole, it appears that duplicate tests made in different batches attain a level of precision which is adequate for most practical purposes. Nevertheless, for some investigations a further improvement may be desirable. The problem of decreasing the variation between batches is presumably that of obtaining more precise control of conditions in the equipment, particularly with respect to long periods of time. Investigations looking towards the solution of this problem are now being undertaken.

It should be noted that the malting test may be subject to an additional error about which the present investigation yields no information. Harrison and Rowland (5) proposed that all samples be steeped for the same length of time but Dickson *et al.* (4) steep all samples to the same moisture content, determining the time required by pilot steeping tests. If the latter procedure is preferable, which it appears to be, then the accuracy with which the set moisture content can be attained in duplicate samples will affect the precision of the malting test. This consideration did not come in question in the present investigation in which only samples of the same lot of barley were used.

Effects of Different Treatments

The treatments studied consist of the eight possible combinations of steeping for 48 or 60 hr., germinating with the chamber at 53° or 54.5° F., and kilning for 52 hr. at 90° to 165° F. or for 36 hr. at 100° to 175° F. The investigation was not undertaken with the object of studying the effects of different treatments on the resulting malt, since information on these is already available (6) and any experienced maltster could have predicted the qualitative if not the quantitative results. Moreover, general conclusions could hardly be based on an investigation made with only one sample of barley. The study was made primarily to determine the capacity of the equipment and method, so far developed, for proving that differences in procedure affect the resulting malt, and particularly their capacity for proving that a change at one stage of the procedure will have different effects depending upon how other parts of the procedure are carried out.

The data for all series are presented in Table IV in mean values for each treatment over all batches. Each figure, therefore, represents the mean of duplicate determinations made on eight malts.

TABLE IV

MEANS, OVER ALL BATCHES, FOR EACH TREATMENT

Determination	Treatments							
	111	112	121	122	211	212	221	222
Extract, %	75.21	75.14	75.27	75.26	75.37	75.22	75.34	75.26
Moisture, %	3.69	3.52	3.72	3.59	3.75	3.57	3.74	3.63
Color, Lovibond units	2.07	2.28	2.04	2.28	2.07	2.30	2.02	2.19
Diastatic power, °L.	142.1	126.8	137.8	122.9	139.2	123.4	133.6	120.4
Permanently soluble nitrogen as % of wort solids	1.50	1.51	1.46	1.47	1.46	1.47	1.42	1.42
Malting loss, %	11.91	11.91	10.79	10.72	10.78	10.62	10.09	9.92
Sprouts, %	5.02	4.95	4.43	4.47	4.58	4.48	4.03	4.02

A better comparison of the effects of the changes in procedure can be obtained by rearranging and condensing the data. The treatments can be arranged in four pairs differing only in steeping procedure (111 and 211, 112 and 212, 121 and 221, 122 and 222), in four pairs differing only in germination procedure, (111 and 121, etc.), or in four pairs differing only in kilning procedure (111 and 112, etc.). From these data, the mean effects, over all treatments, of changes in steeping, germination and kilning procedure, can be calculated as differences between means of four treatments. Data representing these differences are presented in Table V. Those differences which were shown to be significant by statistical analyses have been marked with an asterisk.

TABLE V

DIFFERENCES CAUSED BY CHANGE IN STEEPING, GERMINATION AND KILNING PROCEDURES 1 AND 2

Determination	Steeping, 1 minus 2	Germination, 1 minus 2	Kilning, 1 minus 2
Extract, %	−0.08*	−0.04	0.08*
Moisture, %	−0.04	−0.04	0.15*
Color, Lovibond units	0.02	0.05	−0.21*
Diastatic power, °L.	3.2*	4.2*	14.8*
Permanently soluble nitrogen as % of wort solids	0.05*	0.04*	−0.01
Malting loss, %	0.98*	0.93*	0.09
Sprouts, %	0.44*	0.52*	0.03

* *Statistically significant.*

The data show that extract is not very sensitive to changes at any stage of the procedure; that moisture and color are sensitive only to changes in kilning; that diastatic power and permanently soluble nitrogen are fairly

sensitive to changes in steeping and germination procedure; that diastatic power is very sensitive to changes in kilning procedure; and that malting loss and sprouts are very sensitive to changes in steeping and germination procedure but are not affected by changes in kilning procedure.

On the whole, the effects are small, but the fact that most of them are statistically significant shows that the equipment and malting method are sufficiently precise for investigation of the effects of small changes in the malting process. It is also apparent that the data may be useful in detecting sources of error in a malting test. For instance, if diastatic power can be determined precisely and malting loss cannot, then the fault must be sought in the steeping and germination equipment and not in the kilns.

The investigation demonstrates quite clearly that if the malting test is to be used merely for obtaining an estimate of extract yield, then comparatively crude equipment and methods will serve satisfactorily. On the other hand, if good estimates of diastatic power and malting loss are desired, every precaution will have to be taken to see that the control of the conditions under which the malts are made is precise.

The design of the investigation permits a study of more complicated aspects of the effects of different treatments. The possibility exists that changes in germination or kilning procedure will have different effects depending upon the moisture content to which the barley is steeped, or that changes in kilning will have different effects depending upon the germination procedure used. These so-called interaction effects have been investigated by means of statistical analyses of the data. Only two of the 21 interactions studied (three interactions for each of seven determinations) proved to be significant. Increasing the temperature of the germination chamber from 53° to 54.5° F. increased malting loss by 1.2% when the barley was steeped for 60 hr. and by only 0.7% when it was steeped for 48 hr. Lower temperatures during kilning increased diastatic power by 15.5° L. when the germination chamber was operated at 54.5° F. and by 14.1° L. when the chamber was operated at 53° F.

The study of the interaction effects should be of considerable interest to those working with the laboratory malting test. If numerous, comparatively large, interaction effects existed, considerable difficulty might be anticipated in developing a precise malting test, because errors introduced by variations at one stage of the process would be magnified by variations at another stage of the process. Moreover, if large interaction effects existed, it would be more difficult to investigate the effects of changing the conditions of a single stage of the malting process, because the effects of such changes would be dependent upon the conditions maintained in the rest of the process. In these circumstances simultaneous investigations of the effects of changing several factors would be required, a replication of malting units would be needed, and investigations of a factorial design would have to be undertaken.

The investigation provides some grounds for believing that serious complications of this sort will not arise. Only the interaction of germination

procedure on steeping procedure, with respect to malting loss, is of sufficient magnitude to merit further consideration. The existence of this interaction effect, together with the fact that malting loss is very sensitive to changes in both steeping and germination procedure, suggest that it will be fairly difficult to develop a malting test which is precise with respect to the determination of malting loss.

Statistical Analyses

For each determination, the variance of the data was analyzed into portions due to: (i) variations in the general level of results obtained in different batches; (ii) average differences, over all batches, between treatments; (iii) differences in the relative performance of treatments in different batches; (iv) differences between duplicate malts, and (v) differences between duplicate analyses. The variance due to average differences, over all batches, between treatments, was then analyzed into portions due to (vi) average differences between steeping procedures; (vii) average differences between germination procedures; (viii) average differences between kilning procedures; (ix) the interaction between steeping and germination procedures; (x) the interaction between steeping and kilning procedures; (xi) the interaction between germination and kilning procedures, and (xii) the triple interaction between steeping, germination and kilning procedures. The results of the analyses of variance are summarized in Table VI.

TABLE VI

ANALYSES OF VARIANCE FOR ALL DATA

Variation due to	Degrees of freedom	Mean squares						
		Extract, %	Moisture, %	Color units	Diastatic power, °L	Perm. sol. nitrogen, %	Malting loss, %	Sprouts, %
Treatments	7	.0424**	.0591**	.1139**	567.597**	.00824**	4.3089**	1.0771**
Batches	3	.0782**	.0217	.0203	216.710**	.01497**	2.6004**	.9247**
Treatments × batches	21	.0097	.0088††	.0101††	13.856††	.00048†	.1430††	.0207††
Steeping	1	.1008**	.0264	.0100	165.766**	.02911**	15.4056**	3.0976**
Germination	1	.0324	.0264	.0355	284.766**	.02743**	13.6900**	4.3681**
Kilning	1	.0930**	.3452**	.7225**	3507.601**	.00065	.1406	.0203
Steeping × germination	1	.0315	.0014	.0156	.090	.00033	.8557*	.0042
Steeping × kilning	1	.0204	.0001	.0039	1.434	.00001	.0626	.0086
Germination × kilning	1	.0183	.0126	.0025	8.492*	.00001	.0057	.0410
Steeping × germination × kilning	1	.0002	.0015	.0076	4.529	.00011	.0023	.0000
Duplicate malting tests	32	.0134	.0028	.0038	1.967	.00022	.0084	.0067
Duplicate analyses	64	.0098	.0029	.0048	1.277	.00053		

Double signs denote that the mean square attains a 1% level of significance, single signs denote a 5% level.

* *and* ** *Significantly greater than the mean square due to treatments × batches.*

† *and* †† *Significantly greater than the mean square due to duplicate malting tests.*

The significance of the results of the analyses was determined by application of the Z test and the standard errors, reported earlier in this paper, were calculated from the appropriate mean squares in the usual manner.

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AN INVESTIGATION OF STRAWBERRY VIRUS DISEASE IN ONTARIO[1]

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Abstract

Following identification in 1932 of the Yellow-edge virus disease in England on the Royal Sovereign variety, "normal" plants of this variety from a clone minutely rogued for Yellow-edge were used at St. Catharines as indicators in a further study of virus as relating to certain Ontario varieties. Observations in the field and greenhouse, confined largely to the varieties Parson's Beauty, Premier (Howard 17), Forward and Glen Mary, showed that symptoms analogous to those of Yellow-edge in England and sufficiently defined to permit of diagnosis were apparent only on Parson's Beauty and Forward, and then only for a limited period early in the growing season. In the 1933-35 transmission experiments (by runner grafting), symptoms macroscopically indistinguishable from those of typical Yellow-edge-infected plants in England were induced on Royal Sovereign from the local varieties Glen Mary, Parson's Beauty and Premier, which possess markedly the symptomless-carrier capacity. Of special interest was the deterioration of Premier components in certain graft series, the evidence suggesting reciprocal infection between test and indicator plants.

Finally, parallel experiments at the East Malling Station in 1935–36 provided supplementary data as follows: (1) Of the two parent *Fragaria* species common to commercial varieties in North America and in England, *F. chiloensis* was found to be a symptomless-carrier of Yellow-edge with a high order of resistance, and *F. virginiana*, in complete contrast, exhibited symptoms with extreme readiness together with high susceptibility, thus providing some explanation of the observed wide range of varietal reaction to disease of the Yellow-edge type. (2) A large proportion of the clone of Royal Sovereign plants used as "normal" indicators in the recent series of experiments, was found to be infected with a distinct virus of the "Crinkle" type, thus providing explanation of an observed reciprocal reaction in certain series with the Premier variety.

General Introduction

As the result of investigations begun in 1931 at East Malling, the senior author determined the incidence of a virus disease of strawberries in commercial stocks of the Royal Sovereign variety in southwestern England (3). At that time the close similarity between the disease in England and Xanthosis or Yellows in California (9) was pointed out and the popular descriptive name of Yellow-edge was proposed for the former. Subsequently, a year's residence in eastern Canada, at the Dominion Laboratory of Plant Pathology, St. Catharines, Ontario, from May, 1933 (under an exchange arrangement with Dr. G. H. Berkeley, Senior Pathologist-in-charge of that laboratory) provided the senior author with valuable opportunity for extending his field of reference in the study of virus diseases of the strawberry.

A preliminary survey of experimental and commercial plantations in the Niagara Peninsula was followed up experimentally by attempts to infect "normal" plants of the English indicator variety, Royal Sovereign, (similar in clonal origin to those used in the original East Malling experiments and

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shipped to St. Catharines for the purpose) from plants of local varieties widely grown in Ontario, using the method of grafting (runner-inarching) devised at East Malling (2). Throughout these preliminary studies the senior author had the advantage of collaboration with the junior author, who was at that time commencing a study of a serious form of failure of the root-rot type, in local strawberry plantations (5). The initial small-scale experiments on virus disease yielded results indicating that the incidence of such disease on local varieties was more widespread, and of a more serious nature than was apparent from symptoms visible in the field; and the question arose as to the freedom from virus of the plant material of certain varieties used in the root-rot experiments. At the suggestion of the Dominion Botanist, Dr. H. T. Güssow, therefore, a scheme for future experiments, designed to test and extend the preliminary work of 1933, was drawn up jointly by the present writers, and suitable plant material selected and prepared before the termination of the exchange period. Thereafter the investigation at St. Catharines was carried on by the junior author whilst the senior author resumed his experiments at East Malling on the English-grown varieties, with particular reference to Royal Sovereign.

Early in 1936 an account of the experiments carried out at St. Catharines in 1934 and 1935 was forwarded to East Malling. The results of these were found in the main to confirm the conclusion tentatively reached in 1933 and further to indicate that virus disease in Ontario was more general in occurrence than had been realized.

At the same time complementary results were forthcoming from the further experiments with English-grown varieties at East Malling in 1935, certain of which indicated that the clonal race of Royal Sovereign plants used in the St. Catharines experiments and tentatively regarded as "virus-free" was actually infected with virus, distinct, however, from Yellow-edge virus. This conclusion was amply confirmed by a large-scale experiment in 1936 and provides an explanation of an otherwise anomalous and inexplicable reciprocity in the reaction of certain series of Premier-Royal Sovereign grafts in the 1935 St. Catharines experiments.

In the pages that follow, the share of the investigation borne by each of the writers has been indicated by placing the initials of the worker primarily responsible after the heading of each constituent section of the paper.

Initial Investigations at St. Catharines, 1933-34 (R.V.H.)

A. Field Survey

1. Variety Parson's Beauty

Early in June, 1933, extensive plantations of this variety at St. Davids, Ontario, were examined.* The growers reported a progressive falling-off of vigor and cropping in this stock of plants during the preceding seasons, culminating in the 1933 season when the bulk of the plants were found to be

** The writer accompanied Mr. G. C. Chamberlain, Acting Officer-in-charge of the St. Catharines laboratory, on this visit, and is grateful to Mr. Chamberlain for drawing his attention to the symptoms in question.*

in an advanced stage of deterioration. At this time the majority exhibited leaf symptoms similar to those of Yellow-edge in Royal Sovereign in England, but in no case so clearly pronounced, particularly in the matter of marginal chlorosis.

In all subsequent visits by both writers to these plantations the symptoms were found to have become completely masked and diagnosis impossible. At the time of the first visit, infected plants showing the typical symptoms were selected and transferred to pots in the greenhouse for experimental purposes.

2. Variety Premier (Howard 17)

In June, 1933, Dr. J. H. L. Truscott (11) at the Vineland Horticultural Station drew the attention of the senior writer to a series of plants of this variety recently collected from a local deteriorating plantation. Dr. Truscott stated that attempts to isolate pathogenic fungi from the roots of these plants had given negative results, but that a proportion of the plants showed slight symptoms of the so-called "mosaic" (1) or June Yellows (10) disease. The plants were taken to St. Catharines for further observation and were used in the experiments to be described below. During the subsequent period of observation and experimentation, a proportion of the plants and their progeny showed symptoms of "mosaic" but at no time was any trace of symptoms analogous to those of Yellow-edge (Xanthosis) detected. When the writers visited the plantation of origin with Dr. Truscott in June, 1934, a proportion of the plants were found to show "mosaic" (June Yellows) and mite infestation symptoms, but again it was impossible to find symptoms of Yellow-edge.

This variety is extremely susceptible to root rot (5) and numerous visits were made to plantations affected by this disease. In view of results from the concurrent virus transmission experiments, a special look-out was kept for symptoms of the Yellow-edge type, but except for plants that were found to be "flat" in appearance, with very slightly chlorotic leaves, no sufficiently distinct and clear-cut symptoms were observed in this variety to make approximate field diagnosis or rogueing even remotely possible.

3. Other Varieties

During the 1933 season one of the writers (A.A.H.) carried out extensive experiments with the variety Glen Mary in connection with his root-rot investigations (5). Plants of this variety, in both commercial and experimental plantings, were kept under constant observation for symptoms of Yellow-edge, but no symptoms similar to those previously seen on Parson's Beauty at St. Davids were observed.

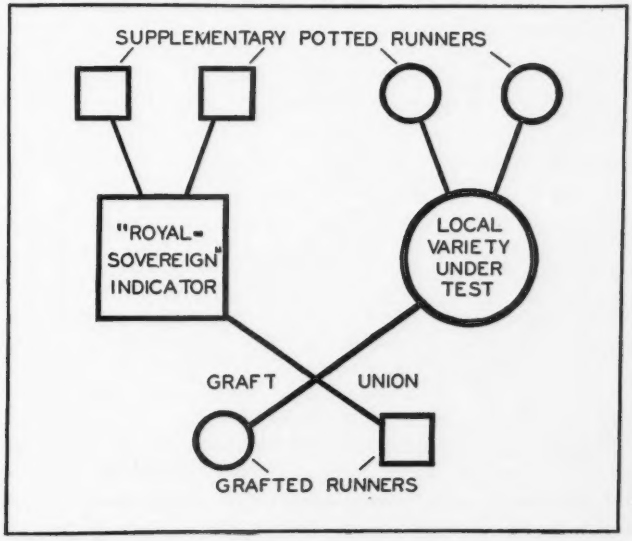
In a small greenhouse population of the variety Forward observed by the senior author for some months after his arrival at St. Catharines, one plant showed symptoms closely analogous to those of Yellow-edge. No runners were produced by this plant or others of the group so that it was not possible to carry out grafts on to Royal Sovereign as indicator.

During visits to the Central Experimental Farm at Ottawa, in June and September, 1933, and July, 1934, surveys were made of extensive collections of seedlings raised by M. B. Davis of the Division of Horticulture. The majority of these were free from distinguishable symptoms of Yellow-edge, but certain of them showed symptoms similar to those seen on Parson's Beauty and Forward.

B. Experimental

1. Material and Methods

All the "normal" Royal Sovereign plants (termed indicators below) used throughout the entire series of experiments at East Malling and at St. Catharines, referred to in this communication, were derived from a clonal family originating from a single plant in 1928 and subsequently subjected continuously to direct measures for pest control and to regular inspections, re-selections, and rogueing to eliminate plants showing lack of health and vigor in general, and the Yellow-edge disease in particular. Of the 50 "normal" indicator plants in the first consignment shipped to St. Catharines in March, 1933, only seven plants survived, but these developed into uniformly vigorous and healthy plants and were ready for grafting early in August. The only impediment to their development was severe infestation with mite (*Tarsonemus pallidus* Bks.) which appeared early in June on all plants in this series, and also on the Forward, Parson's Beauty and Premier series. All plants were therefore subjected to two applications of the warm water treatment (in June and September, 1933, respectively) to control this pest (6).



TEXT-FIG. 1. *Plan of graft-unit*

The method of grafting used was that of runner-inarching as in the East Malling experiments (2) with an improved binding medium substituted for the old raffia and wax, namely, a proprietary sheet form of self-sealing, pure crêpe rubber.* With this material a graft union can be thoroughly sealed and bound rigidly and permanently in place in a single operation.

The general scheme of a graft-unit is shown in Text-fig. 1. In the East Malling 1932 experiments the passage of the virus from infected to healthy plants via the inarched stolons, and thence to all the runner progeny, was found to be extremely rapid and it was, therefore, decided in the present case not to strike into pots the runners on the grafted stolons, but to pot instead at least two ungrafted (supplementary) runners each from the Royal Sovereign indicator plants and from the plants of the local variety under test.

2. Experiments with Parson's Beauty

Of the eight plants showing symptoms analogous to Yellow-edge, collected from St. Davids, only two eventually produced runners suitable for grafting. Two graft-units were set up with these on August 5, each of which made union.

On September 19, at the time of the second warm water treatment, the familiar symptoms of the Yellow-edge type were visible on both Royal Sovereign parent indicator plants in spite of the masking effect of mite, which had completely obscured Yellow-edge symptoms on Parson's Beauty.

On October 30, distinct Yellow-edge symptoms were recorded on all components of the indicator variety in both units (Plate I, Fig. 1). Of the Parson's Beauty plants, both grafted runners showed clear symptoms, one of the parent plants showed symptoms very indistinctly, the other not at all, and no symptoms were recorded on any of the supplementary runners. Thereafter, the symptoms became completely masked on the Parson's Beauty plants but persisted on the indicators. The latter rapidly deteriorated in vigor of growth until March, 1934, when the stunting had become extreme (Plate II, Fig. 1). Ungrafted indicator checks which received the same treatment for mite remained free from Yellow-edge symptoms and developed normally and vigorously throughout (Plate II, Figs. 1 and 2).

3. Experiments with Premier

No symptoms analogous to Yellow-edge were detected at any time on the six plants of this variety obtained from Vineland. Three of them showed the distinct and characteristic symptoms of "mosaic" (June Yellows) transitorily and slightly at the time of transfer to St. Catharines.

Only two plants produced runners suitable for grafting; one of these belonged to the "mosaic" group. Two units were set up with these plants on August 5, 1933, and in both cases union took place.

At the time of the second warm water treatment on September 19, no definite virus symptoms were visible on any of the indicator plants and runners. On October 30, definite symptoms, closely resembling those of Yellow-edge, were recorded on the parent indicator plant and on one of

* *Supplied by the Sterling Rubber Company, Guelph, Ont., under the name of Sterlaid.*

the supplementary indicator runners of one of the units (Plate I, Fig. 2). Later, (unlike the indicators in the Parson's Beauty experiment) the symptoms on these plants became masked and it was not until March, 1934 that they reappeared distinctly. At this time also, and not till then, definite Yellow-edge symptoms were recorded on the parent plant and on supplementary runners of the remaining unit. At no time during the experiment were symptoms of the Yellow-edge (Xanthosis) type recorded on the test (Premier) plants or their runners (Plate II, Fig. 2). Finally, the rate of deterioration of the indicators in the Premier experiment was not so rapid as in the Parson's Beauty experiment (Plate II, Fig. 1).

As has already been recorded in the account of the former experiment, the ungrafted indicator checks and their progeny remained normal and vigorous throughout.

C. Discussion of Results and Conclusions

Of the local varieties studied, symptoms analogous to Yellow-edge on Royal Sovereign were observed only on Parson's Beauty (in the field and in the greenhouse) and on a single plant of Forward (in the greenhouse). These symptoms were at no time as pronounced as those on Royal Sovereign plants in plantations in England or infected from Parson's Beauty in the greenhouse at St. Catharines. Further, the tendency for the diagnostic symptoms to become masked (symptomless-carrier capacity) was found to be much more pronounced in the two local varieties than in Royal Sovereign plants infected from these varieties and kept under identical (greenhouse) conditions. Further, rapid symptom recession or masking took place in the field following the initial record of symptoms on Parson's Beauty in June, 1933, and persisted throughout the remainder of the senior writer's residence in Canada. A similar progressive symptom-masking (particularly in the matter of the marginal leaf chlorosis) was also recorded on the plants of both the above local varieties in the greenhouse, until in October no definite symptoms could be detected, although such were distinctly manifest at the same time on the parallel Royal Sovereign indicator plants.

That the Premier variety possesses the symptomless-carrier capacity to an even more marked degree than Parson's Beauty is suggested by the fact that, although at no time during the investigations were any diagnostic symptoms recorded in any field plantation of this variety or on any single plant in the greenhouse, certain of the latter plants were shown to be infected with virus (of the Xanthosis-Yellow-edge type) by the use of Royal Sovereign plants as indicators.

Thus, it would appear that a survey of the incidence of virus disease on local varieties in Ontario based on macro-symptoms in the field, bears but little relation to the distribution of infected plants.

Although, as noted above, under uniform greenhouse conditions a distinct contrast was recorded (both in degree of clearness and in tendency to masking) between the symptoms shown by Parson's Beauty and Forward, and those

induced in the parallel Royal Sovereign indicator plants (infected from both Parson's Beauty and Premier), on the other hand, the symptoms appearing on the indicators were indistinguishable from those of Yellow-edge occurring naturally or induced artificially on that variety in England. This implies that any difference between the symptomatological pictures in both countries is related primarily to differences inherent in the varieties grown, rather than to wide differences between the pathogens or the environmental conditions of cultivation. Thus the close similarity between Yellow-edge disease in England and the analogous disease in Ontario is emphasized.

Finally, the lack of vigor and the markedly poor performance of the infected plants of both Premier and Parson's Beauty suggest that although these varieties, and particularly the former, possess a capacity for acting as symptomless-carriers (for the Xanthosis-Yellow-edge type of disease) to a degree greatly in advance of Royal Sovereign, they are both also susceptible to the deteriorating action of the disease. That their degree of susceptibility is, however, lower than that of Royal Sovereign is indicated by the relatively rapid deterioration of plants of the latter variety following infection from the former by grafting.

Further Experiments at St. Catharines 1934-35 (A.A.H.)

Material and Methods

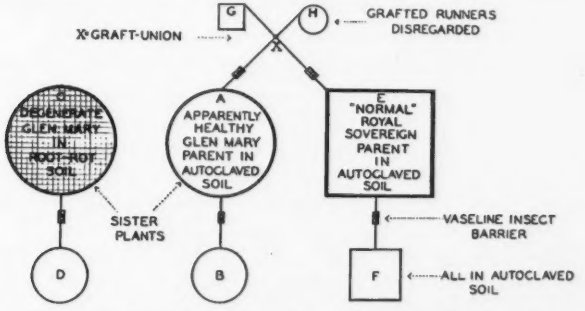
In the experiments carried out by the junior author, attempts at transmission of virus were limited to grafting, the technique of runner-inarching being the same as that already employed by the senior author. In outdoor experiments, however, it was found necessary to supplement the crêpe rubber sealing and binding newly grafted stolons, with an outer wrapping of raffia, to prevent too rapid deterioration of the rubber in direct sunlight.

Attention was concentrated on three local varieties only, namely, Glen Mary, Parson's Beauty and Premier. Stolons from selected specimens of these varieties were grafted to those of indicator plants, variety Royal Sovereign, the clonal origin of which was similar to that of plants used in the same capacity by the senior author in his experiments at East Malling and at St. Catharines. These plants were regarded at the time as virus-free, but as intimated in the general introduction, research in England subsequent to the experiments carried out at St. Catharines showed that this clonal race of Royal Sovereign plants was actually infected with virus, distinct, however, from Yellow-edge. This accounts for repeated reference to these as "normal" rather than as virus-free plants.

In all, 10 different series of grafts involving 61 individual graft-units were completed, some in the greenhouse, others in outdoor plots. Where possible, all component plants of a series were grown in autoclaved soil, thus eliminating the effect of soil organisms as a complicating factor. Facilities were not available for the prevention of infestation of plants by insect pests but, when necessary, remedial measures were adopted, as, for example, periodic warm water treatments for the control of the Tarsonemid mite (6).

Series I *Variety: Glen Mary*

In 1933, as reported elsewhere (5), significant differences in the vigor of growth of runners were obtained by training each member of a series of clonal pairs of runners of the variety Glen Mary, (i) in autoclaved greenhouse compost and (ii) in non-sterilized soil from a plantation seriously affected with root rot. The resultant vigor of growth of the plants in the former was significantly greater, both as regards aerial parts and root systems. These observed differences were found to be correlated with the comparative freedom from fungi and nematodes of the roots of runners grown in the sterilized soil and with an abundance of these organisms in the roots of those grown in the non-sterilized plantation soil. It was decided that it might be interesting to carry the experiment further and test the sets of genetically identical runner plants for the presence or absence of virus. Consequently, on June 8, 1934, the stolons of five apparently healthy Glen Mary plants raised in autoclaved soil in the greenhouse were grafted to those of "normal" Royal Sovereign plants, and on July 8, five similar grafts were made. As they became available, runners from each of the parents were struck in autoclaved soil. Also, as they became available, runners produced by the "degenerate" Glen Mary component of each clonal pair were struck in autoclaved soil. The scheme of grafting and disposition of individual plants comprising a typical graft-unit of Series I is illustrated graphically in Text-fig. 2.



TEXT-FIG. 2. *Scheme of grafting and disposition of individual plants comprising a typical, fully completed Glen Mary × Royal Sovereign graft-unit (Series I).*

The plants involved in the experiment were kept in the greenhouse for almost a year. To afford them full opportunity for development, they were transferred to new soil (autoclaved) in larger pots during the winter, and to preclude the masking effect of mite injury, they were periodically given the warm water treatment. The results appearing in Table I represent the final decisions arrived at, following a series of observations which finally terminated on June 5, 1935, after the plants had passed through the spring period of renewed growth activity.

By July 24, that is, within seven weeks from the time the grafts had been made, indicator plants exhibited typical symptoms of Yellow-edge as evidenced

by (i) chlorosis or yellowing of the marginal region of the leaflets; (ii) an abnormally "flat" appearance, the foliage consisting of a zone of more or less normal outer leaves enclosing a central zone of dwarf or Yellow-edge leaves (Plate III, Fig. 1, E and F). By October the general symptomatological picture had changed, the diagnostic symptoms now chiefly in evidence being a general dwarfing of the plant, an irregular curling of the marginal regions of the leaflets, abnormally short petioles and a more or less general distortion and asymmetrical appearance of the leaves (Plate III, Fig. 2, E and F). In this experiment, once a Royal Sovereign plant became infected, it never subsequently showed other than very limited power of recovery and none of the affected plants produced stolons.

During the last week in July, when the Royal Sovereign runner plants of the graft-units were unmistakably showing symptoms of Yellow-edge, the soil was washed from their roots, which were then compared with the roots of "normal" non-grafted, Royal Sovereign runner plants of approximately the same age (about nine weeks). Macroscopically it was at once apparent that the roots of the Yellow-edge plants lacked the general bulk of those of the healthy, non-grafted plants. Microscopical examination showed that, though the root systems of both grafted and non-grafted plants were not free from organisms—fungi and nematodes,—such organisms were present at this time in such relatively small numbers that they could not be held accountable for the dwarfed condition of the roots of the plants involved in the graft-units.

Complete results as summarized in Table I show (i) that all graft-unions were successful, (ii) that seven of the ten degenerate Glen Mary plants raised in the non-sterilized plantation soil produced runners which when struck in autoclaved soil developed into vigorous, healthy plants, (iii) that all the Glen Mary plants and their runner progeny remained healthy except for insect injury in some cases, and (iv) that all the Royal Sovereign plants and the runner progeny of each developed symptoms indistinguishable from those of Yellow-edge. Before the termination of the experiment, four of the affected Royal Sovereign plants had died (Units 5, 6, 8 and 10), one was dying and the remainder were showing varying stages of deterioration. Like the Glen Mary plants, the Royal Sovereign plants no doubt also suffered injury from insect attack, but, in their more or less degenerate condition, the latter was difficult to distinguish as such and certainly was not of sufficient consequence materially to affect correct interpretation of the results.

Series II

While the results obtained in the greenhouse experiments in 1934 seemed sufficiently conclusive, it nevertheless appeared advisable to reserve final decision until a graft series could be completed under outdoor conditions. Consequently, on July 3, 1935, eight Glen Mary plants, chosen because of their exceptionally healthy and vigorous appearance from a population of this variety growing in outdoor plots, were runner-grafted to "normal" Royal

TABLE I

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY GLEN MARY PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting				
					Glen Mary			Royal Sovereign	
					D	B	A	E	F
					Runner from degenerate parent	Runner	Parent	Parent	Runner
I	1	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy M*	Y.E.*	Y.E.
I	2	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Dying
I	3	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy S*	Y.E.	Y.E.
I	4	8.6.34	Greenhouse	+		Healthy	Healthy	Y.E.	Y.E.
I	5	8.6.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Dead
I	6	8.7.34	Greenhouse	+	Healthy	Healthy S	Healthy MS	Dead	Y.E.
I	7	8.7.34	Greenhouse	+	Healthy	Healthy	Healthy	Y.E.	Y.E.
I	8	8.7.34	Greenhouse	+		Healthy M	Healthy	Y.E.	Dead
I	9	8.7.34	Greenhouse	+		Healthy	Healthy	Y.E.	Y.E.
I	10	8.7.34	Greenhouse	+	Healthy	Healthy	Healthy	Dead	Y.E.

TABLE I—*Concluded*

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY GLEN MARY PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting				
					Glen Mary			Royal Sovereign	
					D	B	A	E	F
					Runner from degenerate parent	Runner	Parent	Parent	Runner
II	11	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.
II	12	3.7.35	Outdoors	−		Healthy	Healthy	Healthy	Healthy
II	13	3.7.35	Outdoors	+		Healthy	Healthy	Healthy	Healthy
II	14	3.7.35	Outdoors	+		Healthy	Healthy	Healthy	Healthy
II	15	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.
II	16	3.7.35	Outdoors	−		Healthy	Healthy	Healthy	Healthy
II	17	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.
II	18	3.7.35	Outdoors	+		Healthy	Healthy	Y.E.	Y.E.

* M = *mite injury;* Y.E. = *"Yellow-edge";* S = *red spider injury.*

Sovereign plants, transferred from the greenhouse in the pots in which they had been raised. As checks, four additional "normal" Royal Sovereign plants were transferred to the outdoor environment. A runner from each parent in a graft-unit was struck in autoclaved soil. Eight graft-units comprised the series.

The Royal Sovereign plants, upon being removed to the outdoor plots, proved to be very susceptible to attack by the leaf-spot organism, *Mycosphaerella fragariae* (Schw.) Lind., the infection becoming so severe as to render it extremely difficult to evaluate the possible effect of any other disease-producing agent. Critical diagnosis of symptoms apart from leaf-spot was therefore not attempted under outdoor conditions. On September 19, all Royal Sovereign plants, both parents and runner progeny, also the runner progeny of the Glen Mary parents, were transferred back to the greenhouse. All leaves affected with leaf-spot were clipped off, the plants were given the warm-water treatment to rid them of possible mite infection and then they were transferred to new soil (autoclaved) in larger pots. The plants then developed relatively free from leaf-spot and mites, and in a series of observations it was possible to evaluate the effect due presumably to grafting alone.

As reference to Table I will show, all but two of the graft-unions were successful. It seems significant that in the two cases of failure (Units 12 and 16) the indicator Royal Sovereign plants remained healthy. Of significance also, is the fact that in two cases where the grafts *were* successful, symptoms of Yellow-edge did not appear in the Royal Sovereign components (Units 13 and 14). In the remaining four cases symptoms characteristic of Yellow-edge developed in the Royal Sovereign plants (Units 11, 15, 17 and 18). The four check plants, though attacked outdoors by leaf-spot and possibly by mites, recovered later in the greenhouse and did not show symptoms of Yellow-edge.

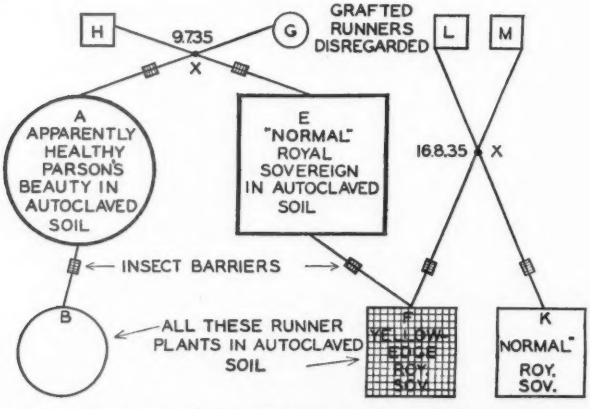
Discussion Regarding the Variety Glen Mary

Following successful grafting of the stolons of Glen Mary plants selected because of their apparent health and vigor, to those of "normal" Royal Sovereign plants, 14 parent plants (out of a possible 16) of the latter variety, and runner progeny from each, developed symptoms indistinguishable from those of Yellow-edge as described on the English-grown variety. This may be taken as almost conclusive evidence that plants of the Ontario-grown variety are symptomless carriers of the disease. That this capacity is associated with a high degree of resistance to this particular disease is shown by the fact that Glen Mary plants from which the disease was transmitted to the indicator variety, continued to manifest the outstanding vigor of growth that is characteristic of the variety, both in the greenhouse and in an outdoor environment. Since, following two successful graft-unions, the indicator Royal Sovereign plants remained unaffected, the inference would be that some Glen Mary plants have not become infected with (the) virus.

Series III *Variety: Parson's Beauty*

In the fall of 1933 the present investigators inspected the plantation at St. Davids where, earlier in the season, the senior author had obtained suspect plants, variety Parson's Beauty, from which he was successful in transmitting Yellow-edge to "normal" Royal Sovereign plants (4). On the occasion of the second inspection, no plants could be found that exhibited characteristic symptoms of Yellow-edge, other than some that presented an abnormally "flat" appearance. A number of these were transferred to the greenhouse and in the spring of 1934 runners produced by them were struck in autoclaved soil. These runners developed into plants apparently healthy and true to variety. They were kept under observation until July 9, on which date seven of them were runner-grafted with "normal" Royal Sovereign plants.

In each of three graft-units of this series a runner-plant from the grafted Royal Sovereign (indicator) parent was itself runner-inarched to a further ("independent") "normal" Royal Sovereign indicator. The scheme of grafting of the three fully-completed units of the series is illustrated graphically in Text-fig. 3.



TEXT-FIG. 3. *Scheme of grafting and disposition of individual plants comprising a typical fully completed Parson's Beauty × Royal Sovereign graft-unit (Series III).*

Reference to Table II will show (i) that all graft-unions were successful, (ii) that all but two of the Parson's Beauty plants, parents and runner progeny, remained healthy except for injury by insects in certain cases, and for apparent lack of vigor in two plants (Units 21 and 23), which, however, displayed no symptoms suggestive of any specific disease of strawberry known to the writer, (iii) that all the Royal Sovereign parent plants with one doubtful exception (Unit 22), and the progeny of the five that *did* produce runners, developed symptoms indicative of Yellow-edge and (iv) that the three Royal Sovereign runner plants to which Yellow-edge had been transmitted through

TABLE II

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PARSON'S BEAUTY PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft-series	Graft-unit No.	Date of graft	Location of experiment	Result of graft-union	Condition of plants subsequent to grafting				
					Parson's Beauty		Royal Sovereign		
					B	A	E	F	K
					Runner	Parent	Parent	Runner	Independent
III	19	9.8.34	Greenhouse	+		Healthy	Y.E.*		
III	20	9.8.34	Greenhouse	+	Healthy	Healthy S*	Y.E.	Y.E.	Y.E.
III	21	9.8.34	Greenhouse	+	Healthy	Abnormal	Y.E.		
III	22	9.8.34	Greenhouse	+	Healthy	Healthy	Doubtful	Y.E.	Y.E.
III	23	9.8.34	Greenhouse	+	Abnormal	Healthy M*	Y.E.	Y.E.	Y.E.
III	24	9.8.34	Greenhouse	+	Healthy	Healthy M	Y.E.	Y.E.	
III	25	9.8.34	Greenhouse	+	Healthy	Healthy	Y.E.	Y.E.	

* Y.E. = "*Yellow-edge*"; S M = *injury by red spider and mite, respectively.*

their respective parents from the apparently healthy Parson's Beauty plants, in turn, transmitted the disease when grafted back to the three independent "normal" Royal Sovereign plants (Units 20, 22 and 23).

Discussion Regarding the Variety Parson's Beauty

Since "normal" Royal Sovereign plants, subsequent to being runner-grafted to apparently healthy Parson's Beauty plants, developed typical symptoms of Yellow-edge, it is evident that plants of the latter variety, like those of the variety Glen Mary *can* be symptomless-carriers of (the) virus of Yellow-edge. That symptoms of the disease may, however, be manifest in plants of the variety is indicated by Harris' original discovery of Yellow-edge in Parson's Beauty plants in Ontario (4).

Series IV *Variety: Premier*

Because of the interesting and significant results that had already been obtained by the senior author in his experiments involving plants of the Premier variety, it was deemed advisable at the time to extend the investigations to include a larger number of plants of this widely grown variety. In the fall of 1933, the present investigators inspected a plantation of Premier plants where, during the summer, root rot had been severe. Thirty plants were brought back to the laboratory, 20 being chosen as apparently healthy, the other ten being selected because of their subnormal vigor and somewhat "flat" appearance, the latter condition being considered as possibly indicative

of virus infection. These plants were potted in fertile soil, and during the ensuing winter they received special attention. After the period of renewed growth activity in the spring, it became evident that only a few of the plants could be regarded as "normal". The majority of them developed an abnormally large number of leaves, smaller than those of "normal" plants, which were supported on long, slender petioles. In general, they bore considerable resemblance to Zeller's illustration of plants affected with Witches'-broom (12, p. 332, Fig. 3). Eventually, only three of the original 30 plants produced runners suitable for grafting, and curiously enough, these three plants were a part of the number that had been originally chosen as suspects. One of the three could be regarded as a "normal" Premier plant, while the other two exhibited slight traces of the abnormalities described above. At no time did these or any of the remaining 27 plants exhibit symptoms at all suggestive of Yellow-edge. These three plants were runner-grafted to "normal" Royal Sovereign plants on August 13, 1934, the three units comprising Series IV.

Referring to Table III, it will be noted that all Premier plants involved in Series IV, three parents and two runners, are recorded as healthy. Two of the parents (Units 26 and 27) were those which, before the grafts were made, had shown the Witches'-broom effect mentioned above. Since these two plants remained in very much the same condition subsequent to grafting and showed no symptoms at all suggestive of Yellow-edge, they are recorded as healthy. One of these plants, when runner-grafted to Royal Sovereign, produced no effect on the latter (Unit 26); the other, however, apparently had some effect on the Royal Sovereign component of the graft-unit, though the interpretation of the symptoms was doubtful. The runner in this unit developed symptoms of Yellow-edge. The results in connection with Unit 28 are interesting. This Premier parent was really the only "normal" vigorous plant of the series, yet within ten weeks after the grafts were made, both the Royal Sovereign parent and its runner showed typical and conspicuous symptoms of Yellow-edge, while the Premier and its runner were still in the state of health and vigor shown in Plate IV, Fig. 1.

The above small-scale experiments indicated that (i) the Witches'-broom effect, whatever its cause or nature, was not transmitted from Premier to Royal Sovereign; (ii) all Premier plants are not infected with (the) virus of Yellow-edge, and (iii) an apparently healthy Premier plant may be a symptomless-carrier of (the) virus of Yellow-edge.

Series V

When, early in 1934, it became apparent that the 30 plants mentioned above in connection with Series IV were not going to provide sufficient material for further grafting, runners being produced by Premier plants in an outdoor plot at the laboratory were struck in autoclaved soil in pots, June 3, 1934. Three weeks later, June 24, the young runner plants were transferred to the greenhouse, where they were kept under close observation for a month. During this period they made vigorous growth and produced robust stolons. On July 25, the stolons of seven of these Premier plants which

could not be considered as other than healthy, vigorous plants, were grafted to those of "normal" Royal Sovereign plants. Included in Table III are the summarized results of a series of observations extending from August, 1934, until after the period of renewed growth activity in the spring of 1935.

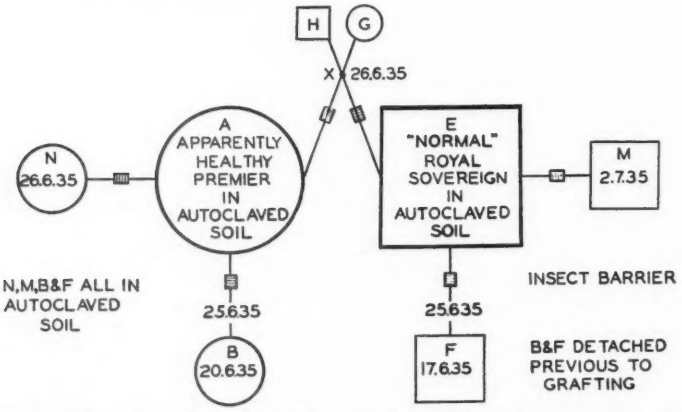
The results obtained in Series V were entirely different from those observed in any of the other series up to that point. Whereas in other experiments Royal Sovereign components alone had shown adverse effects subsequent to grafting, in this case Premier plants showed even more striking effects than did Royal Sovereign. Within a few weeks after grafting, symptoms of abnormality were already in evidence in certain of the Premier plants. The larger leaves began to die, the outer, older ones first, and then successively those formed later. Numerous new leaves were formed but these were small and were supported on short, variously twisted and distorted petioles, the combined effect giving a plant a dwarfed, "bunchy" appearance. At stages in the decline of the Premier plants the foliage showed discoloration and chlorosis but the chlorosis was not localized in the marginal regions of the undersized leaves. Since there was apparent in the Premier plants a gradation in severity of effect, a disease rating was assigned to each plant as shown in Table III, one plus sign indicating an almost healthy plant, while five denote a dead plant. It will be noted that two of the Premier plants died (Units 32 and 35). These plants having been raised in autoclaved soil, their death is all the more surprising since mortality of plants in the greenhouse even in root-rot soil has been exceptional in the experience of the writer. Microscopic examination of the roots of the Premier runner plants, made soon after the symptoms of abnormality had become apparent in the above-ground parts, showed that micro-organisms were not present in sufficient numbers to be held accountable for the condition of the plants. Further, whereas the roots of these Premier runners of the graft-units had ramified relatively sparsely through the soil, those of non-grafted Premier runners of approximately the same age, also grown in autoclaved soil, had formed solid, compact masses of intertwining roots to such an extent that the plants had become "pot-bound".

All the Royal Sovereign plants, except those in Unit 29, developed symptoms typical of those of plants affected with Yellow-edge. In the exceptional case the plants showed an abnormal condition, but the symptoms were not definitely those of Yellow-edge. The other Royal Sovereign plants, like the Premier components, showed differences in severity of effect and even within a given unit there were not necessarily correspondingly severe manifestations of disease on the part of all components (Units 32 and 35).

Series VI

In view of the unusual results obtained in Series V in 1934, it was decided to repeat the experiment. Consequently, as early as possible in June 1935, runners being produced outdoors by the Premier plants which had furnished the plants for Series V, were struck in autoclaved soil in pots. These were later transferred to the greenhouse, and after developing into robust plants as

their predecessors in 1934 had done, were runner-grafted to "normal" Royal Sovereign plants. As far as possible, parent plants which were producing at least three stolons were chosen for the series. Previous to grafting, one stolon from each parent was struck in autoclaved soil. Following detachment of these runner plants from their respective parents, the grafts were made using a second stolon from each plant, then subsequent to grafting, a third runner from each parent was struck in autoclaved soil. The date of grafting, the ages of the various components relative to this date, and the general disposition of the individual plants comprising a graft-unit of Series VI are shown in Text-fig. 4. Six graft-units were involved in the series and the results as finally interpreted are included in Table III.



TEXT-FIG. 4. *Scheme of grafting and disposition of individual plants comprising a typical, fully completed Premier × Royal Sovereign graft-unit (Series VI).*

All six grafts were successful. Before the termination of the experiment, the Premier parent plants became almost uniformly degenerate plants. In marked contrast with these, the runners detached from the parents previous to grafting developed into healthy plants, thus proving that the conditions which obtained in the greenhouse throughout the experiment were not of themselves detrimental to the growth of plants of this variety. The runners struck in autoclaved soil subsequent to the grafts being made, and left attached to the parent plants, consistently developed a condition intermediate between that of the healthy detached runners and the degenerate parent plants (Plate IV, Figs. 2 and 3). In Table III, their condition is recorded as abnormal with a two-plus disease rating.

All six Royal Sovereign parents developed symptoms of Yellow-edge as did also the plants developed from runners left attached after the grafts had been made. In this series, as in Series V, a gradation in severity of effect was noted and the plants were assigned a disease rating as shown in Table III. Of the six runners struck in autoclaved soil and detached from the parent

TABLE III

RESULTS OBTAINED IN GREENHOUSE EXPERIMENTS FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft series	Graft unit	Date of graft	Result of graft union	Condition of plants subsequent to grafting									
				Premier					Royal Sovereign				
				N	B		B		E		F		M
				Runners detached previous to grafting	Runners left attached to parents	Disease rating	Parents	Disease rating	Parents	Disease rating	Runners left attached to parents	Disease rating	Runners detached previous to grafting
IV	26	13.9.34	+				Healthy		Healthy		Healthy		
IV	27	13.9.34	+		Healthy		Healthy		Doubtful of interpretation		Y.E.		
IV	28*	13.9.34	+		Healthy		Healthy		Y.E.		Y.E.		
V	29	25.8.34	+		Abnormal	+++	Degenerate	+++	Doubtful of interpretation		Doubtful of interpretation		
V	30	25.8.34	+		Degenerate	+++	Degenerate	+++	Y.E.	+++	Y.E.	+++	
V	31	25.8.34	+		Degenerate	++	Degenerate	++	Y.E.	+++	Y.E.	+++	
V	32	25.8.34	+		Dead	+++++	Almost dead	++++	Y.E.	++	Y.E.	++	
V	33	25.8.34	+		Degenerate	++	Degenerate	++	Y.E.	+	Y.E.	+++	
V	34	25.8.34	+		Fairly healthy	+	Fairly healthy	+	Y.E.	++	Y.E.	++	
V	35	25.8.34	+		Dead	+++++	Almost dead	++++	Y.E.	++	Y.E.	++	

* *This unit photographed. See Plate IV, Fig. 1.*

TABLE III—*Concluded*

RESULTS OBTAINED IN GREENHOUSE EXPERIMENTS FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft unit	Date of graft	Result of graft union	Condition of plants subsequent to grafting									
				Premier					Royal Sovereign				
				N	B		B		E		F		M
				Runners detached previous to grafting	Runners left attached to parents	Disease rating	Parents	Disease rating	Parents	Disease rating	Runners left attached to parents	Disease rating	Runners detached previous to grafting
VI	36	26.6.35	+	Healthy	Abnormal	++	Degenerate	+++	Y.E.	+++	Y.E.	+	Y.E.
VI	37	26.6.35	+	Healthy			Degenerate	+++	Y.E.	++	Y.E.	+	Healthy
VI	38	26.6.35	+	Healthy	Abnormal	++	Degenerate	+++	Y.E.	+++			Healthy
VI	39	26.6.35	+	Healthy	Abnormal	++	Degenerate	+++	Y.E.	++	Y.E.	++++	Healthy
VI	40	26.6.35	+	Healthy			Degenerate	++	Y.E.	++++	Y.E.	+	Dead**
VI	41	26.6.35	+	Healthy	Abnormal	++	Degenerate	++	Y.E.	++			Healthy

** *Died as result of being detached too soon from parent.*

plants previous to grafting, four remained healthy, one died (Unit 40, detached from the parent too soon), and one developed symptoms of Yellow-edge (Unit 36). The occurrence of the disease in the latter plant might be explained on the basis either of the parent plant already having become infected (though not showing symptoms) before the runner was detached, or of transmission of the virus from one of the numerous infected plants in the greenhouse, by an agent at present unknown.

Series VII–X

While the above-mentioned experiment was in progress in the greenhouse, another series of grafts was made under outdoor conditions. Early in the spring of 1935, Premier plants had been obtained from sources in Ontario as widely separated as Norfolk, Elgin, Wentworth and Lincoln Counties. These were planted in outdoor plots where they were kept under close observation. Later, during the period of prolific runner production, apparently healthy, vigorous plants were runner-grafted to "normal" Royal Sovereign plants, the latter being transferred from the greenhouse in the pots in which they had been raised. A runner from the parent of each variety was struck in autoclaved soil. Four series, VII to X inclusive, each series involving Premier plants from a different source and altogether comprising 20 graft-units, were completed within the period June 24–July 16, 1935.

As had been the case in a similar outdoor experiment (Series II above), the Royal Sovereign plants, soon after being removed to the outdoor environment, became so severely infected with strawberry leaf-spot that it was almost impossible to evaluate the possible effect of any other disease-producing agent. However, during the course of the summer certain of the leaves of the Royal Sovereign components of the graft-units showed most conspicuous marginal chlorosis. Early in September, all Royal Sovereign plants, also the runner progeny of the Premier plants, were transferred to the greenhouse where, after being treated as described for Series II, the plants developed relatively free from leaf-spot and injury by mites.

As reference to Table IV will show, only four of the grafts failed to make union. In two cases where the grafts were successful (Units 43 and 44), the Premier plants remained continuously healthy; the Royal Sovereign parents exhibited symptoms doubtful of interpretation but their runner progeny remained healthy. In two additional cases where the grafts had been successful (Units 56 and 61), the Premier components remained healthy while the Royal Sovereign plants developed symptoms of doubtful interpretation. In the remaining 12 cases of successful graft-unions, all the Premier plants and nine of their runner progeny remained healthy, whereas the 12 Royal Sovereign parents and the runner progeny of each developed symptoms indicative of the presence of Yellow-edge. The disease was transmitted from at least two Premier plants obtained from each of the four different localities. Judging from the severity of effect on the Royal Sovereign components, it would appear that the Premier plants obtained from Wentworth and Elgin Counties were more heavily infected with virus than those from Norfolk and

TABLE IV

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS OBTAINED FROM DIFFERENT SOURCES WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS

Graft series	Graft unit	Date of graft	Result of graft union	Location of experiment	Source of Premier plants	Condition of plants subsequent to grafting					
						Premier		Royal Sovereign			
						B	A	E		F	
						Runner	Parent	Parent	Disease rating	Runner	Disease rating
VII	42	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Y.E.	++	Y.E.	+
VII	43	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Doubtful		Healthy	
VII	44	24.6.35	+	Outdoor plots	Norfolk County	Healthy	Healthy	Doubtful		Healthy	
VII	45	24.6.35	+	Outdoor plots	Norfolk County	Abnormal	Healthy	Y.E.	+++	Y.E.	+++
VII	46	3.7.35	–	Outdoor plots	Norfolk County	Healthy	Healthy	Healthy			
VII	47	3.7.35	–	Outdoor plots	Norfolk County	Healthy	Healthy	Healthy			
VIII	48	2.7.35	+	Outdoor plots	Lincoln	Healthy	Healthy	Y.E.	++	Y.E.	++
VIII	49	2.7.35	+	Outdoor plots	Lincoln	Healthy	Healthy	Y.E.	++	Y.E.	++
VIII	50	2.7.35	–	Outdoor plots	Lincoln	Healthy	Healthy	Healthy		Healthy	
VIII	51	2.7.35	–	Outdoor plots	Lincoln	Healthy	Healthy	Slightly abnormal		Healthy	
IX	52	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	+++	Y.E.	+
IX	53	3.7.35	+	Outdoor plots	Wentworth	Abnormal	Healthy	Y.E.	++	Y.E.	++++

TABLE IV—*Concluded*

RESULTS OBTAINED FOLLOWING GRAFTING OF STOLONS OF APPARENTLY HEALTHY PREMIER PLANTS OBTAINED FROM DIFFERENT SOURCES WITH THOSE OF "NORMAL" ROYAL SOVEREIGN PLANTS—*Concluded*

Graft series	Graft unit	Date of graft	Result of graft union	Location of experiment	Source of Premier plants	Condition of plants subsequent to grafting					
						Premier		Royal Sovereign			
						B	A	E		F	
						Runner	Parent	Parent	Disease rating	Runner	Disease rating
IX	54	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	+++	Y.E.	+
IX	55	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	++		
IX	56	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Doubtful			
IX	57	3.7.35	+	Outdoor plots	Wentworth	Healthy	Healthy	Y.E.	++	Y.E.	++
X	58	16.7.35	+	Outdoor plots	Elgin	Abnormal	Healthy	Y.E.	++	Y.E.	+
X	59	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Y.E.	+++	Y.E.	++
X	60	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Y.E.	+		
X	61	16.7.35	+	Outdoor plots	Elgin	Healthy	Healthy	Doubtful		Doubtful	

Lincoln Counties. In regard to the plants obtained from Norfolk County, it is worthy of mention that correlated with their apparent "milder" infection with virus was their outstanding vigor of growth.

Discussion Regarding the Variety Premier

Altogether, 36 Premier-Royal Sovereign runner-grafts were made, 16 in the greenhouse all successful, and 20 outside, four of the latter being unsuccessful. *It is significant that in all four cases of failure of graft-union (Units 46, 47, 50 and 51), the components of each variety remained healthy.*

In three cases of successful graft-unions (Units 26, 43 and 44), all Premier components remained healthy and none of the Royal Sovereign plants involved developed symptoms suggestive of Yellow-edge. The evidence in regard to the three present cases would suggest that the Premier parents were not infected with the virus of Yellow-edge and that inter-varietal "incompatibility" can be ruled out as a complicating factor in observed results.

In three other cases of successful graft-unions (Units 29, 56 and 61) the Royal Sovereign components all developed symptoms doubtful of interpretation, that is, the plants could not be regarded as normal; yet the symptomatological picture they presented could not be associated with any of the diseases of strawberry known to the writer. In one of the three cases under consideration (Unit 29, greenhouse), the Premier parent and its runner raised in autoclaved soil both became degenerate plants, but in the other two cases (Units 56 and 61, outdoors), the Premier parents and a runner from one of them remained healthy. The results obtained in these three cases suggest that virus other than that of Yellow-edge may be present in certain Premier plants.

In the remaining 26 cases of successful graft-unions, 12 outdoors and 14 in the greenhouse, symptoms of Yellow-edge developed in all the Royal Sovereign components. The results in regard to the Premier components are especially interesting. With two exceptions (Units 27 and 28), all Premier plants involved in greenhouse graft-units became more or less degenerate plants, as did also the runner progeny in Series V. In Series VI, the runners, which certainly were abnormal plants, did not approach the same degree of degeneracy. As sharply contrasted with results obtained in the greenhouse, all 12 Premier parents involved in the outdoor graft-units remained healthy, as did the runner progeny of nine of them, even after being transferred to the greenhouse in September. With regard to the anomalous decline of the Premier plants in Series V and VI the following explanations might be postulated. (i) The principal factor may be environmental, these experiments having been carried out in the greenhouse and the subsequent series (VII to X) in the open air. Against this it must be noted that the initial small-scale experiment in 1933 was carried out in the greenhouse and its results, as regards the behavior of the Premier plants, corresponded closely to those of the open air series (VII to X). Again, when the runner progeny of Premier parents, which in outdoor graft-series had transmitted Yellow-edge to Royal Sovereign plants, were transferred to the greenhouse they remained

in a healthy condition. Then, too, in Series VI, Premier runner plants detached from parent plants previous to grafting remained healthy, while at the same time, under identical conditions, sister plants left attached to the parents after the grafts had been made showed marked adverse effects while the parent plants themselves became degenerate plants. (ii) Up to this point the Royal Sovereign indicator plants have been assumed to be virus-free and therefore unlikely to have any reciprocal reaction on plants of the local test varieties runner-inarched to them. The anomalous behavior of the grafted Premier plants of the series in question would be comprehensible on the assumption that the indicator plants, although Yellow-edge-free, were infected with another pathogen (virus) and therefore had a reciprocal action on the test plants. That such was actually the condition of the indicator plants has been shown by the subsequent experiments with the Royal Sovereign clone at East Malling in 1935 and 1936, the results of which will be summarized in the following section of this paper.

Another point of interest as affecting Premier-Royal Sovereign grafts is the apparent gradation in severity of effect, especially in the series carried out in the greenhouse in which the effects appeared to be reciprocal. A glance at Table III will show that, as regards the Premier components, the range in effect is from fairly healthy (Unit 34) to dead plants (Units 32 and 35). This would suggest that all Premier plants are not infected to the same degree. Yet, if this were true, it would seem only logical to expect that there would be correspondingly slight or severe manifestations of disease on the part of both test and indicator plants of a given graft-unit. This is certainly not the case in Units 32, 35 and 40.

Both in outdoor and in greenhouse experiments all the Premier parent plants were kept under observation for some time before grafting, and then only those which gave every appearance of health and vigor were chosen. Yet in 26 cases, Royal Sovereign plants runner-grafted to these apparently healthy Premier plants developed the symptomatological picture associated with the Yellow-edge disease. Further, the disease was transmitted from at least two Premier plants obtained from each of four different localities in Ontario. It would appear, therefore, that not only are Premier plants symptomless-carriers of the virus of Yellow-edge, but that infection of plants of this variety is widespread in Ontario.

Further Experiments at East Malling, 1935-36* (R.V.H.)

In 1935 the scope of the experiments on the symptomatology of Yellow-edge was extended to include species of *Fragaria* and cultivated varieties additional to Royal Sovereign, using the latter variety both as a standard source of infection and as an indicator.

In these experiments plants of the varieties under test (test plants) were combined with Royal Sovereign in two types of graft-unit. (1) Test plants

* *A detailed account of these experiments is in course of preparation for early publication. Here, the results are summarized in so far as they have a bearing on the results of the St. Catharines experiments.*

were runner-inarched to "normal" Royal Sovereign indicators in order to determine possible infection in the absence of symptoms (symptomless-carriers). (2) Yellow-edge Royal Sovereign plants were runner-inarched to test plants and these again to "normal" indicators, in order to obtain comparative data on symptom expression in the variety under test. The results of these experiments to date, in so far as they have bearing on the present investigation, are briefly summarized below.

A. The Parent *Fragaria* Species of the Cultivated Strawberry

1. *Fragaria chiloensis* proved to be a symptomless-carrier of Yellow-edge. All of the large series of test plants used in this experiment were found to be Yellow-edge infected, and at no time has any trace of symptoms of this disease been recorded on any of the plants. The vigor of growth of all the plants (not markedly great at the beginning), has been uniformly maintained to the present time.

2. *Fragaria virginiana*, in complete contrast to the above, proved to be extremely susceptible to the disease in all respects. All the test plants were found to be free from infection at the start, but on being infected from Royal Sovereign they developed the complete range of Yellow-edge symptoms distinctly. These symptoms persisted continuously, at periods when such were masked on the Royal Sovereign indicators, and the infected plants rapidly succumbed.

B. Cultivated Varieties Other than Royal Sovereign

The five leading commercial varieties selected for the initial experiment were found to form a susceptibility series between the two extremes described above. At the *F. chiloensis* end came Lefebvre, which approximated to this species closely in high symptomless-carrier capacity and in high (although not absolute) resistance. Towards the *F. virginiana* end (high symptom expression and susceptibility), came Sir Joseph Paxton and at the extreme end, Royal Sovereign itself.

The initial stages of the experiment were carried out in the greenhouse, but from the fall of 1935 throughout 1936 the complete series was transferred to the open air. The *relative* varietal reaction to Yellow-edge remained the same under both sets of conditions.

C. "Crinkle" at East Malling on Royal Sovereign, and *F. vesca* as an Indicator

In 1934, Ogilvie, Swarbrick and Thompson (8) published a description of symptoms observed on Royal Sovereign in the western districts of England, answering closely to Zeller and Vaughan's descriptions of the virus disease "Crinkle" in the Pacific northwestern states (13).

Shortly after the writer's return from Canada (in August, 1934) similar symptoms were found at East Malling on seedlings of certain crosses of English and American varieties, under field test. In collaboration with the Long

Ashton Horticultural Research Station, attempts to transmit the symptoms (by runner-inarching), from these seedlings and from seedling type-material supplied by Long Ashton, yielded positive results. At a later stage, however, all the indicator plants in this experiment developed definite Yellow-edge symptoms, superimposed to varying degree on the "Crinkle" symptoms. The distinct nature of the "Crinkle" disease, however, became evident from results of an experiment in infection with Yellow-edge of the common woodland strawberry, *Fragaria vesca*. Normal *F. vesca* plants runner-inarched to Yellow-edge Royal Sovereign rapidly developed symptoms typical of Yellow-edge. In addition to these symptoms, however, a distinct blistering of the leaves, not observed in similar infections of other species and varieties, was recorded on the test plants. On the other hand, contrary to expectation, similar ("Crinkle" type) symptoms, *but uncombined with the Yellow-edge symptoms*, developed on a single *F. vesca* plant inarched to a "normal" (Yellow-edge-free) Royal Sovereign indicator plant, suggesting the infection of the latter with a disease distinct from Yellow-edge. On close examination of all available Yellow-edge and "normal" Royal Sovereign plants, small and generally very inconspicuous groups of minute circular chlorotic lesions were found, invariably on the former and on a large proportion of the latter.

In 1936, a large-scale confirmatory experiment was carried out testing *F. vesca* as an indicator of Yellow-edge and "Crinkle". A considerable number of "normal" Royal Sovereign plants derived from the clonal family supplying the indicators in the present investigation, were runner-inarched singly to "normal" *F. vesca* plants. Clearly marked and in many cases severe symptoms of the "Crinkle" type, entirely uncomplicated by those of the Yellow-edge type, subsequently developed on 78% of the latter. On the other hand, up to the present (winter 1936–37) all the Royal Sovereign plants have remained uniformly "healthy", vigorous, and normal in appearance, with the exception of the aforementioned inconspicuous groups of minute chlorotic spots. In other clones of Royal Sovereign, distinct in origin from that used in the above experiments, a proportion of the *F. vesca* indicators developed clearly marked Yellow-edge symptoms in addition to the "Crinkle" symptoms.

It has thus become evident that in addition to Yellow-edge, a further distinct virus disease ("Crinkle") is widely, and probably invariably, combined with the Yellow-edge disease in Royal Sovereign, and is widely distributed, generally in "mild" and inconspicuous form, throughout the main Yellow-edge-rogued, "normal" clonal family, and other "healthy" stocks of this variety at East Malling.

Making allowance for a probable spread of infection during the short period between the shipments to St. Catharines and the above experimental survey, it can be assumed, therefore, that the majority of the indicator plants used in the 1934–35 experiments (V, above) and probably in the 1933 experiments were infected with "mild Crinkle".

The great majority of clonal "normal" Yellow-edge-free Royal Sovereign plants hitherto observed in the field, and all plants used as indicators in

the greenhouse, have shown "Crinkle" only in the "mild" and comparatively innocuous form, *i.e.*, the plants are uniformly vigorous and normal, except for inconspicuous groups of minute circular lesions detectable only on close examination. "Serious" cases as illustrated by Ogilvie, Swarbrick and Thompson (8) were identified in 1935 and more commonly in 1936, their distribution in plantations being quite sporadic. That the "serious" phase of "Crinkle" is the result of a re-infection of plants with a further virus pathogen, *i.e.*, that "Crinkle" is complex in origin, is indicated by the results of experiments in 1935, when the "serious" phase of "Crinkle" was artificially induced on Royal Sovereign plants infected with "mild Crinkle" by inarching such plants (i) with seedling varieties infected with "serious Crinkle" plus Yellow-edge, and (ii) with plants of the Stirling Castle variety proved to be free from Yellow-edge and with no visible symptoms of "Crinkle". In the former instance, Yellow-edge as well as "serious Crinkle" was transmitted to the "mild-Crinkle"-infected indicators. Such results provide further evidence (1) that Yellow-edge and "Crinkle" are distinct in origin and (2) that "serious Crinkle" is induced by the interaction of two or more viruses.

General Discussion

The results obtained in the present investigations have already been discussed in considerable detail. The following general considerations and conclusions, with particular reference to the bearing of the results of the recent experiments at East Malling on those of the St. Catharines experiments call, however, for review.

Contrary to what might have been expected from field surveys of symptoms, the experiments with three commercial varieties grown in Ontario have further indicated the close similarity of the Yellow-edge disease in southeast England to the analogous disease in southern Ontario, and have provided considerable evidence in favor of their possible identity. The relationship, however, between either of these diseases and Xanthosis in California, has not yet been experimentally determined so that, pending further detailed and experimental comparisons of the three diseases, they must merely be regarded as common members of a well defined group of virus diseases.

It has further been shown that the three local varieties in question, Parson's Beauty, Premier (Howard 17), and Glen Mary (and to an outstanding degree the last two varieties) possess markedly the "symptomless-carrier" capacity, and that consequently the paucity or the entire absence of diagnostic symptoms in field plantations of these varieties in Ontario does not imply that the incidence of the Xanthosis-Yellow-edge type of disease is slight or that the disease does not occur.

Experimental evidence further indicates that the apparent contrast in the general symptomatological picture in Ontario and in southeastern England is related primarily to inherent differences in susceptibility between the prevalent varieties in the two countries and to differences in cultural conditions mainly in so far as such determine the varieties selected for local cultivation.

The recent experiments at East Malling (1935) on the reaction to Yellow-edge of varieties other than Royal Sovereign, have further shown that this contrast in the symptomatological pictures is not, generally speaking, as great as was suggested when Royal Sovereign was the sole respresentative of the English varieties available for comparison. Certain widely grown varieties such as Lefebvre and Oberschlesien were found to exhibit a high resistance and symptomless-carrier capacity comparable to that of the three Ontario varieties investigated at St. Catharines.

The close association of high resistance to the action of Yellow-edge with the symptomless-carrier capacity was further shown by these experiments to be referable to the contrasted reaction to the disease of the two parent species, common to both the English and Canadian cultivated strawberry varieties, namely *F. chiloensis*, which up to the present has proved to be a perfect symptomless-carrier of Yellow-edge with a markedly high degree of resistance which may prove to be absolute, and *F. virginiana*, which combines very high susceptibility with continuous and vivid symptom expression. The relative degree to which such varieties as Premier, Glen Mary, Lefebvre and Oberschlesien approximate to *F. chiloensis* in resistance to the deteriorating action of virus is a subject for future investigation. Certain of these varieties, however, have shown clearly that their high resistance is not absolute and as these varieties do not appear to exhibit diagnostic symptoms sufficiently clearly to allow of an adequate elimination of infected plants in propagating beds, and thus of the maintenance of Yellow-edge-free stocks, their survival in commerce would appear to be of limited duration.

Finally, the recent experimental evidence, (a) of a wide infection of the Yellow-edge-free Royal Sovereign indicator plants used in the present investigation with a "mild" form of disease distinct in origin from Yellow-edge and conforming closely to descriptions of virus disease of the "Crinkle" type, and (b) the complex origin of the "serious" phase (by re-infection of a "mild Crinkle" infected plant) provides an explanation of the anomalous behavior of the Premier test plants in graft-series V and VI. It would appear that the race of Premier plants used in these series, *i.e.*, that raised at the St. Catharines laboratory was, in addition to Yellow-edge, infected with a further virus which in combination with the "mild Crinkle" virus contributed by the Royal Sovereign indicators, causes the former plants to pass into a condition of rapid deterioration. Verification of this hypothesis must await further work in the analysis of the virus complex in strawberries; meanwhile the explanation of the deterioration of the Premier plants in question as due primarily to a reciprocal infection of these plants from the "mild Crinkle"-infected Royal Sovereign indicators is the simplest yet available.

It yet remains to investigate the difference in virus content between the Premier plants in Series V and VI, and those of the previous and subsequent series, a difference which must be assumed if the above hypothesis is accepted. Meanwhile further evidence is provided to show that stocks of Premier runners from different sources may, in spite of a general uniformity of appearance, differ considerably in virus content.

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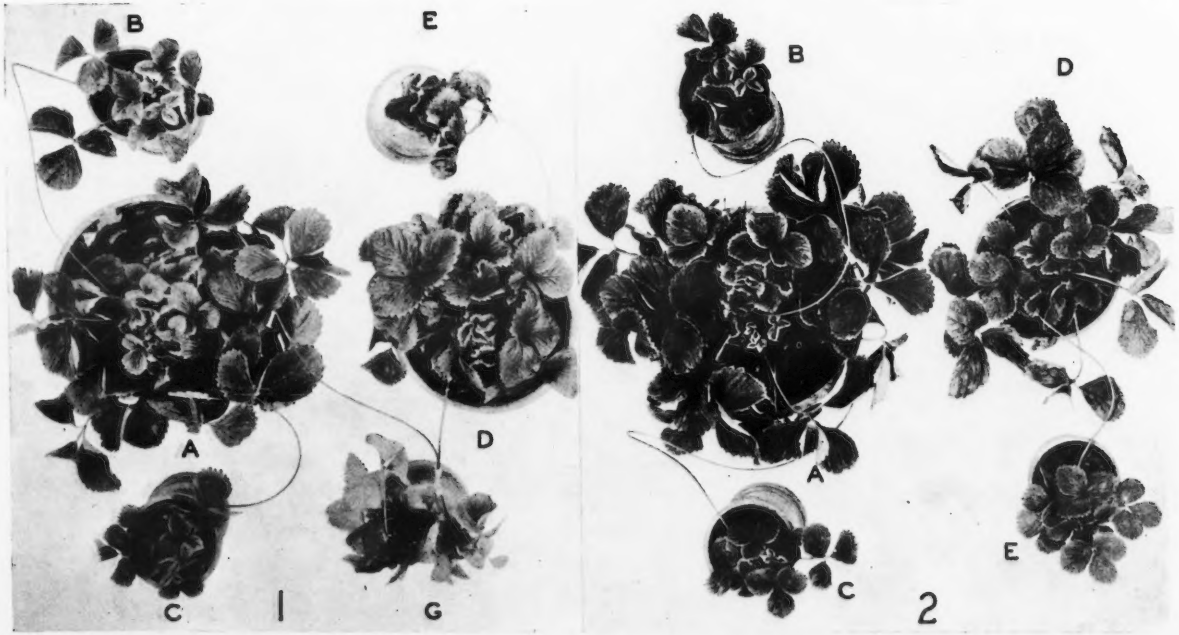
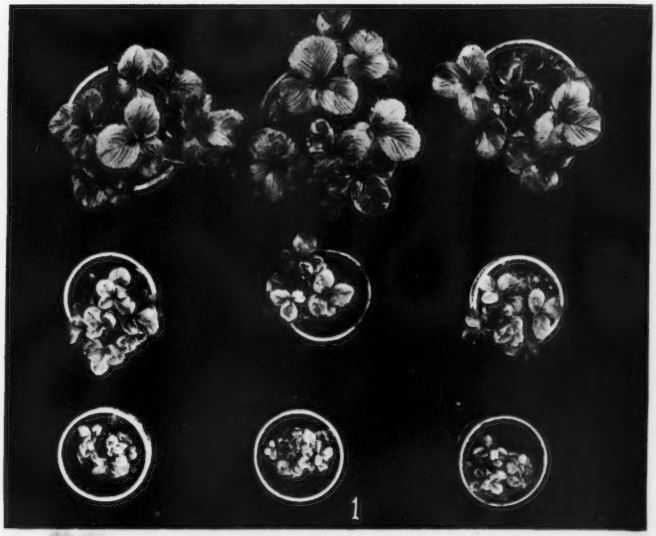


FIG. 1. *Graft-unit of variety Parson's Beauty set up 5.8.33. Photo. 30.10.33. A, Indicator plant with B, C, supplementary runners. D, Test plant with E, supplementary runner. G, Grafted stolons showing point of union.* FIG. 2. *Graft-unit of variety Premier set up 5.8.33. Photo. 30.10.33. Lettering as above. Grafted stolon previously removed.*

PLATE 1



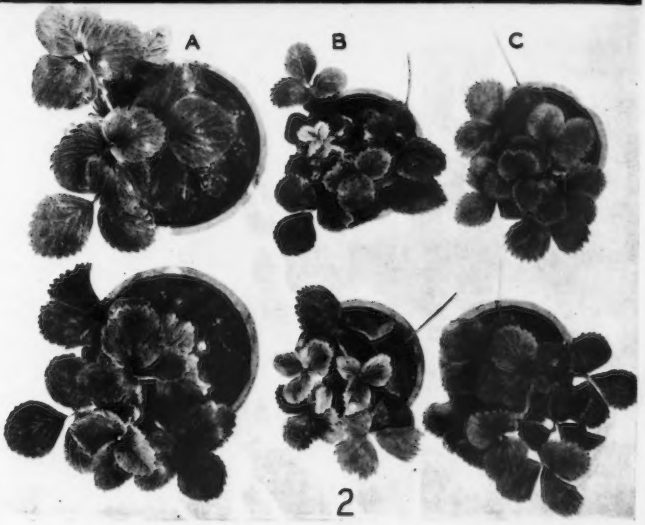
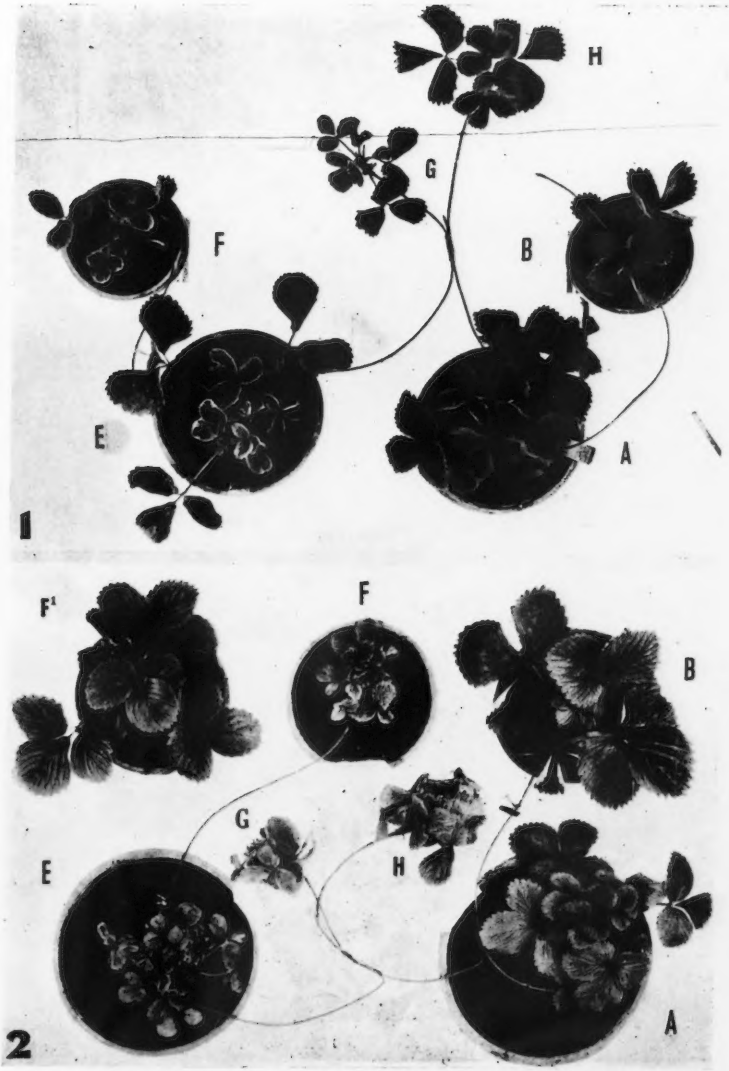


FIG. 1. *Royal Sovereign runner plants. Top row, from ungrafted controls. Middle row, from Premier graft-units. Bottom row, from Parson's Beauty units. Photo. 9.3.34.*
FIG. 2. *Runner plants. A, Royal Sovereign from ungrafted control. B, Royal Sovereign from Premier graft-units. C, Premier from the same graft-units.*



FIGS. 1 AND 2. *Glen Mary × Royal Sovereign graft-unit showing the condition of the component plants six and one-half weeks (Fig. 1) and four and one-half months (Fig. 2) subsequent to grafting. A, B and H, Glen Mary parent, runner in autoclaved soil and grafted runner, respectively. E, F, F' and G, Royal Sovereign parent, runner in autoclaved soil, non-grafted sister-runner of F (approximately the same age) in autoclaved soil and grafted runner, respectively.*

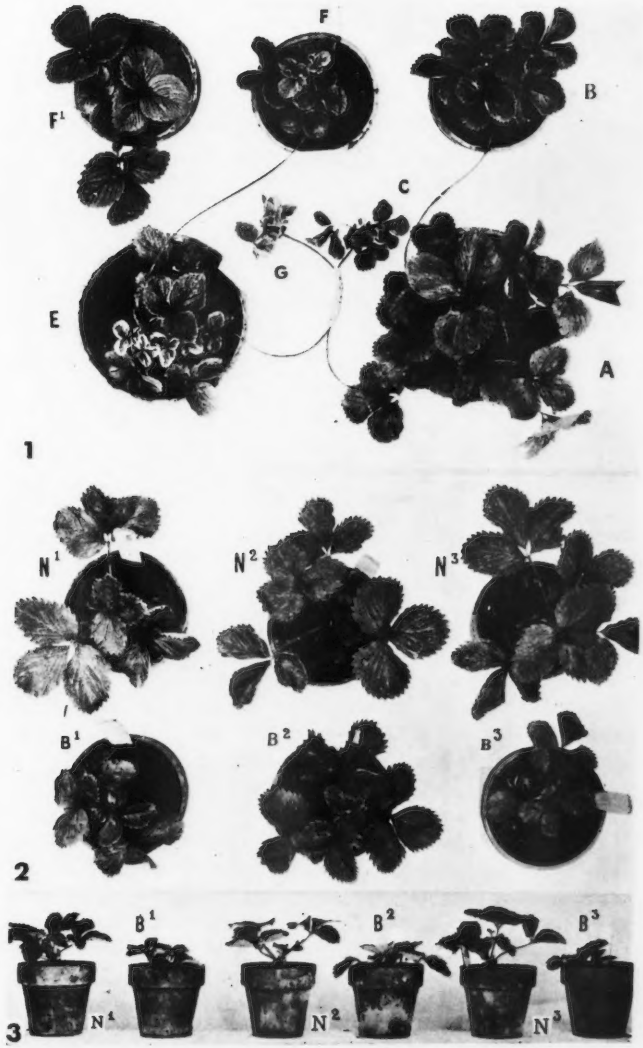


FIG. 1. *Premier × Royal Sovereign graft-unit showing the condition of the component plants ten weeks subsequent to grafting. A, B and C, Premier parent, runner in autoclaved soil and grafted runner, respectively. E, F, F′ and G, Royal Sovereign parent, runner in autoclaved soil, non-grafted sister-runner of F (approximately the same age) in autoclaved soil and grafted runner, respectively.* FIGS. 2 AND 3. *Surface and lateral views, respectively, of genetically identical pairs of Premier runner plants, the "N" components of each pair having been detached from the parent previous to the latter being runner-grafted with Royal Sovereign, the "B" component of each pair having been left attached to the parent subsequent to grafting.*

A CYTOLOGICAL STUDY OF THE GENUS *POA* L.[1]

By J. M. Armstrong[2]

Abstract

The somatic chromosome numbers of 20 species of *Poa* were determined. The basic chromosome number for the genus was found to be seven. The species arranged themselves in a polyploid series from diploid to dodecaploid, tetraploids and hexaploids being the most numerous. Three aneuploid species possessed chromosome numbers suggestive of a nonaploid origin. Polymorphism was found to be present in *P. compressa* L., *P. palustris* L. and *P. nemoralis* L. All species examined conformed to the long chromosome type common to the subfamily, Pooideae. The spindle fibre attachment for the chromosomes in the various species ideograms was found to be regularly median or submedian.

The chromosome variability and the mode of seed production were examined in *Poa pratensis* L., using selected, uniform strains, indigenous plants and plants grown from commercial seed. The somatic chromosome number was found to range from 50 to 87 ± 1, 10 of the 19 plants examined possessing aneuploid numbers. The selected strains possessed the same chromosome number for both plants examined, while in the other material the number was variable. A study of meiosis in the P.M.C. showed the selected strains to vary from regular behavior to an irregularity of 3.9 unpaired univalents per cell. All strains possessed large percentages of morphologically good pollen which germinated actively on the stigmas. Reduction was observed in the E.M.C. of the selected strains and a study of the course of embryological development showed no irregularities which might lead to aposporous reproduction. A high frequency of polyembryony was observed which was correlated to the degree of irregularity at meiosis. A theory is advanced to explain how constant aneuploid numbers may be maintained in sexually reproduced strains.

1. SOMATIC CHROMOSOMES

Poa belongs to the large tribe Festuceae of the Gramineae. The genus has a wide distribution, from moist tropical conditions through every type of habitat in temperate regions to alpine conditions in mountain ranges. There are about 200 recognized species. These species are known as meadow grasses in Britain and as bluegrasses in America. They are mostly perennials but there are a few annuals. Some are stoloniferous while others are of the bunch grass type. While *Poa* is regarded taxonomically as one of the primitive genera of grasses, it presents many difficulties in classification.

Cytologically the genus has not been very extensively investigated. Eleven species were examined by Stahlin (14), nine by Avdulov (2, 3) and six by Müntzing (9). Allowing for repetition, 12 species in all have been examined. This was sufficient to establish the polyploid nature of the genus, with seven as the basic chromosome number. The existence of polymorphic species, characterized by different chromosome numbers for the different biotypes has also been discovered. Müntzing investigated in considerable detail the polymorphous species, *P. alpina*, and to a lesser extent *P. pratensis*. Biotypes were studied which possessed constant aneuploid chromosome numbers, which the author believed were due to asexual seed formation.

Twenty species of *Poa* were examined in the present study. These include 11 not hitherto reported upon. It was thought that a cytological examination

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Contribution from the Division of Forage Plants, Dominion Experimental Farms, Ottawa, Canada.

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of this group, fairly representative of the genus as regards ecological distribution, would enable some conclusions to be drawn as to the nature and origin of polyploidy in the genus. The possibility of apomictical seed formation in *P. pratensis* has also been investigated. The report of this investigation will be dealt with in a separate section of this paper.

Materials and Methods

The plant material used was taken from the introduction nursery of the Division of Forage Plants, Central Experimental Farm, Ottawa, where the economic possibilities of various species are being investigated. The original source of the material is given in Table I. The correct naming of the various species was determined by the Division of Botany, Central Experimental Farm, and in the case of certain European introductions by the Royal Botanic Gardens, Kew, England.

The somatic chromosome studies have all been made on root tips of plants grown in the greenhouse. These plants were taken as clones from plants in the nursery, which in turn were established from seed. Root tips were fixed in La Cour's (6) 2 B. E. fixative. Sections were cut 12 μ thick and stained by Newton's iodine gentian violet method. Drawings were made with the aid of a camera lucida, 30 $\times$ ocular and Leitz objective 1.5 mm., N. A. 1.3, giving a magnification at table level of approximately 6400 $\times$. This has been reduced in reproduction to approximately 2150 $\times$.

Observations

In Table I, a list of the species examined, their source and chromosome number is given. The nomenclature of species native to or introduced to America is that given by Hitchcock (5).

TABLE I

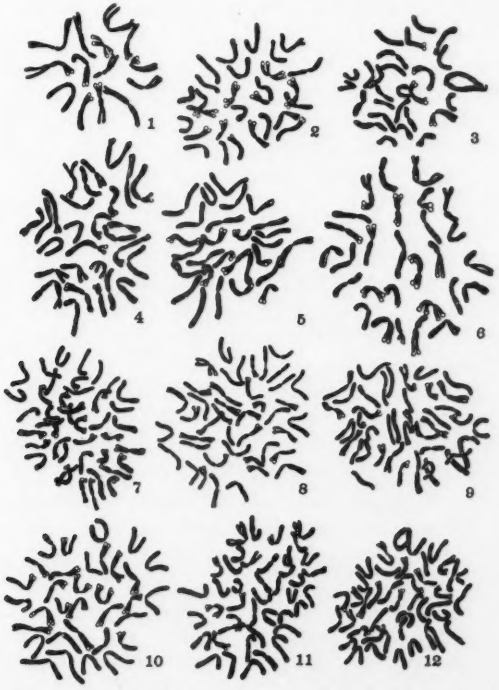
List of species examined, source and somatic chromosome numbers

Species	Source	No. of plants examined	Chromosome numbers
P. trivialis L.	Vilmorin Andrieux, Paris	2	14
P. palustris L.	Vilmorin Andrieux, Paris	2	28
P. palustris L.	University of Manitoba	2	28
P. annua L.	Vilmorin Andrieux, Paris	2	28
P. bulbosa L.	Woronesh, U.S.S.R.	2	28
P. badensis Haenke.	Woronesh, U.S.S.R.	2	28
P. macrantha Vasey	Soil Erosion Service, U.S.A.	2	28
P. nemoralis L.	Sutton & Sons, England	2	42
P. compressa L.	Ewings Seed Co., Montreal	2	42
P. compressa L.	Woronesh, U.S.S.R.	2	56
P. ochroleuca Stern.	Woronesh, U.S.S.R.	2	42
P. alpina L.	Woronesh, U.S.S.R.	3	32 – 34
P. bodryoides L.	Woronesh, U.S.S.R.	2	42
P. sterilis L.	Woronesh, U.S.S.R.	2	42
P. conferta Blyth	Woronesh, U.S.S.R.	2	56
P. epilis Scribn.	Soil Erosion Service, U.S.A.	2	56
P. confusa Rydb.	Dr. S. E. Clarke, Manyberries, Alberta	4	62
P. ampla Merr.	Soil Erosion Service, U.S.A.	2	64
P. nevadensis Vasey	Soil Erosion Service, U.S.A.	2	62
P. arctica R. Br.	Dr. O. M. McConkey, Ontario Agr. College	2	70
P. scabrella (Thurb.) Benth.	Soil Erosion Service, U.S.A.	2	84

P. trivialis L. Introduced from Europe to America where it is now widely distributed in moist situations. The plants examined were from a European commercial seed source and proved to be diploid, $2n = 14$ (Fig. 1). This count was also obtained by Stahlin, Avdulov and Müntzing. *P. chaixii* L. (= *P. sudetica* Haenke.) is the only other diploid species so far identified by the above investigators.

P. palustris L. Hitchcock considers that this species is probably indigenous to both Europe and America, since it is widely distributed in both continents. It is a loosely tufted species found in moist habitats. Plants from two sources were examined cytologically and both proved to be tetraploids, $2n = 28$ (Fig. 2). This agrees with the count obtained by Avdulov. Stahlin, however, reported a hexaploid, $2n = 42$. While *P. palustris* is generally regarded as a "good" species, it shows considerable morphological variability, and polymorphic forms, differing in their chromosome numbers, apparently exist.

P. annua L. This annual species was introduced into America from Europe. The material examined was from a European source. It proved



FIGS. 1–12. FIG. 1. *Poa trivialis L., 2n = 14.* FIG. 2. *Poa palustris L., 2n = 28.* FIG. 3. *Poa annua L., 2n = 28.* FIG. 4. *Pos bulbosa L., 2n = 28.* FIG. 5. *Poa badensis Haenke., 2n = 28.* FIG. 6. *Poa macrantha Vasey 2n = 28.* FIG. 7. *Poa nemoralis L., 2n = 42.* FIG. 8. *Poa compressa L., 2n = 42.* FIG. 9. *Poa ochroleuca Stern., 2n = 42.* FIG. 10. *Poa alpina L. 2n = 33.* FIG. 11. *Poa bodryoides L., 2n = 42.* FIG. 12. *Poa sterilis L., 2n = 42.*

to be a tetraploid $2n = 28$ (Fig. 3). This number agrees with that found by Stahlin and Avdulov.

P. bulbosa L. Introduced from Europe to America where it has become widely distributed. Its distinguishing morphological feature is the bulblets which are found in place of normal flowers. Plants from one source were examined and proved to be tetraploid, $2n = 28$ (Fig. 4).

P. badensis Haenke. (*P. alpina* L. var. *badensis* Koch). A European species introduced from the U.S.S.R. This species proved to be a tetraploid, $2n = 28$ (Fig. 5). Stahlin reports $2n = 42$ for this species.

P. macrantha Vasey. A dioecious species, indigenous to the Pacific Coast, its habitat being the saline sand dunes along the coast. This species is a tetraploid, $2n = 28$ (Fig. 6).

P. nemoralis L. Introduced from Europe to America and now widely distributed in meadow habitats. Our material was obtained from an English commercial source and proved to be hexaploid, $2n = 42$ (Fig. 7). This number agrees with that observed by Stahlin. Avdulov, however, found a tetraploid form and Müntzing an octoploid. Biotypes apparently exist in this species, which show intraspecific variation in chromosome number, probably of autopolyploid origin, since the different biotypes are not taxonomically distinct.

P. compressa L. Introduced from Europe, according to Hitchcock, although it is commonly known as Canada bluegrass. It is characterized by strongly flattened stems. It thrives on certain heavy, clay soils in western Ontario and is often the dominant species on light sandy soils of poor fertility. Our material was obtained from two sources. That from a local commercial source proved to be hexaploid, $2n = 42$ (Fig. 8), while that introduced from the U.S.S.R. proved to be octoploid.* Stahlin and Müntzing found their material to be hexaploid while Avdulov reported an octoploid. Polymorphism is apparently present in this species also, although the autopolyploid origin is not so definite as in *P. nemoralis*, since the variability in the polymorphic forms is more marked.

P. ochroleuca Stern. var. *submoniliformio* Makino. Obtained from Woronesh, U.S.S.R., but the taxonomic description could not be traced by the Royal Botanic Gardens, Kew. In many respects the species closely resembles *P. nemoralis* L. It proved to be a hexaploid, $2n = 42$ (Fig. 9).

P. alpina L. Indigenous to the whole northern hemisphere, its habitat outside of the Arctic regions being mountain meadows. Three plants, examined cytologically from a European introduction, possessed somatic chromosome numbers of 32, 33 and 34 (Fig. 10). Stahlin reported a biotype which had $2n = 42$. Müntzing found quite a complex situation in the Swedish

** Mr. W. Dore, taxonomist at the Botany Division, Central Experimental Farm, Ottawa, has the following to say about this form: "This is a most peculiar plant in that the florets are devoid of any trace of pubescence such as is typical of this species. Every one of the 23 Canadian specimens and four Scandinavian specimens I have examined is typical in being appressed-pilose on the keel and marginal nerves of the lemma, with webby hairs at the base. In this specimen the lemmas are entirely glabrous (except for a slight scabiosity on the upper part of the keel). It is similar to typical P. compressa with regard to all the other characters, and I do not think it can be any other species."*

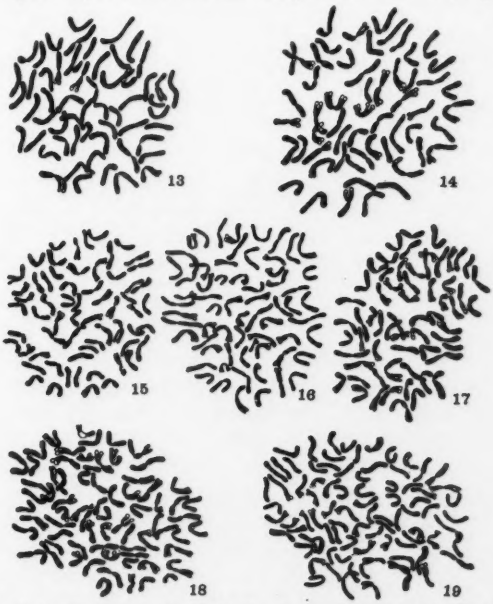
and Swiss biotypes which he examined. Two Swedish biotypes had constant aneuploid chromosome numbers of 33 and 38 and showed a high degree of morphological uniformity within each biotype. The Swiss biotypes, on the other hand, were found to be quite variable in the chromosome numbers, ranging from 22 to 34, and displayed a high degree of morphological variability. Müntzing considered the latter to be unstable sexual types and the former to be reproduced apomictically. The plants in our material varied considerably in vigor and this fact, in conjunction with the variation in chromosome number, indicates that this form is probably an unstable sexual type.

P. bodryoides L. Introduced from Woronesh, U.S.S.R. but has not been identified with certainty by our taxonomists. It proved to be a hexaploid, $2n = 42$ (Fig. 11).

P. sterilis L. Introduced from Woronesh, U.S.S.R. and its correct naming is still uncertain. The plants examined proved to hexaploid, $2n = 42$ (Fig. 12).

P. conferta Blyth. Introduced from Woronesh, U.S.S.R. The plants examined were octoploids, $2n = 56$ (Fig. 13).

P. epilis Scribn. Found in the Pacific Coast area, its habitat being mountain meadows above the timber line. It is characterized by a condensed ovoid panicle. The species proved to be an octoploid, $2n = 56$ (Fig. 14).



FIGS. 13–19. FIG. *13. Poa conferta Blyth., 2n = 56.* FIG. 14. *Poa epilis Scribn., 2n = 56.* FIG. 15. *Poa confusa Rydb., 2n = 62.* FIG. 16. *Poa ampla Merr., 2n = 64.* FIG. 17. *Poa nevadensis Vasey., 2n = 62.* FIG. 12. *Poa arctica R. Br., 2n = 70.* FIG. 19. *Poa scabrella (Thurb) Benth., 2n = 84.*

P. ampla Merr. Occurs in the Pacific Coast area in meadows and rocky slopes from New Mexico to the Yukon territory. Typical forms are robust and more or less glaucous. The chromosome number of the plants examined was aneuploid, $2n = 64$ (Fig. 16).

P. confusa Rydb. While Rydberg gives *P. confusa* a species ranking, Hitchcock considers it to be a form of *P. ampla*. It is common on the eastern part of the range of *P. ampla* and is distinguished from it by being smaller and non-glaucous. The four plants examined (collected by Dr. S. E. Clarke, Manyberries, Alta.) proved to be aneuploid, $2n = 62$ (Fig. 15). Cytologically it is not quite identical with *P. ampla* although the chromosome number suggests the same origin.

P. nevadensis Vasey. Range of distribution from the Yukon territory south to California with the greatest concentration in Montana and eastern Washington. A small isolated colony has also been discovered in Maine. Cytologically it was found to be aneuploid, $2n = 62$ (Fig. 17).

P. arctica R. Br. Range of distribution from Arctic regions south to Nova Scotia and found also on the slopes of the Rocky Mountains above the timber line where the ground is bare of snow only three months in the year. Our material was found to be decaploid, $2n = 70$ (Fig. 18).

P. scabrella (Thurb.) Benth. Occurs in meadows and open woodlands at low to medium altitudes at the Pacific coast. It is distinguished morphologically by the scabrous character of culms and leaves. Aside from certain biotypes of *P. pratensis*, it proved to have the highest chromosome number ($2n = 84$) of any species examined (Fig. 19).

Discussion

In recent years there has been a wide application of cytology to systematics. This has been due largely to improved methods of fixation and staining, permitting a closer observation of chromosome detail. The chromosome characters of value in application to systematics are basic chromosome numbers, polyploidy, chromosome length, the position of the spindle-fibre attachment and secondary features such as the presence of heads and trabants.

Lewitsky (7) has ably reviewed the use of the karotype in systematics. The author uses the term, karyotype, to denote a complex of nuclear characters keeping its significance now over individual, then over race, species, genus, etc. He designates the graphical representation of a karyotype as an ideogram. Separate species would be expected to have characteristic and constant ideograms, and the ideograms of all species within a genus should possess certain features in common. The author points out the danger of too rigid a karyotypic interpretation since closely related species taxonomically may differ widely in their ideograms, owing to the operation of chromosome translocation, and conversely species widely separated taxonomically may show a fairly close similarity in their ideograms. But on the whole a parallelism of karyotypical and external plant characters holds.

The basic chromosome number of the genus *Poa* is seven, which is the most common basic number not only for the tribe Festuceae but for the Gramineae as a whole. Most of the species examined are even multiples of this basic number and arrange themselves in a polyploid series from $2x$ to $12x$ (where x is the basic number). The frequency distribution for the series is one diploid, six tetraploid, five hexaploid, three octoploid, one decaploid and one dodecaploid.

The species, *P. ampla*, *P. confusa* and *P. nevadensis*, constitute exceptions to regular polyploidy. Their aneuploid chromosome numbers of 62 and 64 suggest that they are derivatives from a hybrid nonaploid ($2n = 63$) and have become stabilized by the loss or duplication of a chromosome. If these aneuploid numbers are constant for the various species (this may be presumed to be the case for *P. confusa* in which four plants were examined) either the meiotic behavior is fairly regular, or the chromosome constancy is due to the operation of apomixis, as Müntzing has suggested for certain biotypes of *P. alpina* and *P. pratensis*. Opposed to this hypothesis is the well known difficulty in classification of a group of western Poas comprising such species as *P. canbyi*, *P. nevadensis* and *P. confusa*. This difficulty may be due to hybridization within the above species or to a high degree of heterozygosity. Such difficulties in classification would not be met with if the species were reproduced apomictically.

The situation in *P. alpina* has been fully discussed by Müntzing, and similar biotypes to the one which we have examined were studied by Müntzing and Avdulov.

Next in importance to basic chromosome number and polyploidy in applying karyotology to systematic studies is chromosome length. Frequently tribes in a family are characterized by a common diminution in chromosome length. For example the subfamily Panicoideae, in the Gramineae, has small short chromosomes in comparison to the other subfamily Pooideae, in which the chromosomes are comparatively large and long (Avdulov (2)). Analogous to this situation is the difference in chromosome length in the tribes Vicieae and Trifolieae in the Leguminosae. The former is characterized by large chromosomes and the latter by small ones. The genus *Poa* conforms to the long chromosome type common to the subfamily Pooideae. Some variation in chromosome length exists throughout the various species ideograms of the series. The shorter chromosomes are approximately one-half the length of the longer chromosomes, with others intermediate in length.

The spindle fibre attachment for the chromosomes in the various ideograms of the genus *Poa* is in general median or sub-median. Occasionally chromosomes were observed in which the attachment is subterminal (Figs. 1, 4). Lewitsky (7) on the basis of his study of the Helleboreae concluded that there has been a progressive shortening of one arm in many of the chromosomes, primitive members of group having more isobrachial chromosomes and derived members, mostly heterobrachial ones. Since there is no marked increase in the proportion of heterobrachial chromosomes in the higher polyploids of the

genus it would indicate that their origin, whether due to auto- or allopolyploidy, from lower polyploids in the series, has stimulated very little secondary differentiation such as might be brought about by chromosomal interchange. This conclusion, however, must be regarded as tentative until meiotic studies are completed.

Concerning the origin of the higher polyploids of the genus *Poa*, we are inclined to believe that both auto- and allopolyploidy have taken place in different instances. In *P. nemoralis* and *P. compressa* the existence of polymorphic forms with different chromosome numbers has been noted. As these forms were not sufficiently different taxonomically to give species ranking, the higher polyploid in the species probably originated from the lower in an autopolyploid manner. In the case of decaploid and dodecaploid species, their origin was more likely due to allopolyploidy—the hybridization of lower members of the series, followed by chromosome doubling. The manner of origin and even the mode of reproduction in the aneuploid species whose chromosome numbers approach $2n = 63$ cannot be deduced without a careful study of their meiotic and embryological processes.

II. CYTOLOGICAL VARIABILITY AND MODE OF SEED PRODUCTION IN *POA PRATENSIS* L.

The chromosome conditions in *P. pratensis* have been investigated and reported by several authors. Stahlin (14) published the number $2n = 56$. Avdulov (2) found the somatic numbers 28, 56 and 70. Nakajima (10) counted $2n = 70$. Müntzing (9) determined the chromosome numbers in eight biotypes, mostly of Swedish origin, and reported aneuploid chromosome numbers ranging from 64 ± 1 to 85 ± 1. One heptaploid biotype ($2n = 49$) possessed that number in 10 individual plants examined. In preliminary studies on meiosis in this biotype the author observed the occurrence of irregularities which would lead to the formation of gametes with variable chromosome numbers. Rancken (11) examined four plants from a Finnish biotype and found the numbers to be aneuploid, varying between 66 and 67 + 2ff. In studies of reduction divisions in the P.M.C. this author also observed multivalent chromosome groups and lagging univalents. Müntzing drew the conclusion, with which Rancken is in agreement, that certain biotypes of *P. pratensis* form their seed apomictically. They offer as proof of apomixis the following conditions: (i) an aneuploid chromosome number which is constant for the biotype, (ii) the occurrence of irregularities at meiosis of the P.M.C., (iii) morphological constancy within the biotype, and (iv) good seed production.

Embryological investigations in *Poa pratensis* have been made by Anderson (1) and Nishimura (see (1)). Anderson observed the frequent occurrence of two embryo sacs within the same ovary. The author found the additional embryos to be formed from one of the three, usually nonfunctional, megaspores or from a separate megaspore mother cell. In no case did she observe additional embryos arising agamosporously. Nishimura found

that embryonic buds may arise from the antipodal nucellar region which may supplant the normal embryo. He regards these embryos of sporophytic origin as being the result of galls formed by insect attack.

In the present study plants from a wide range of material have been examined. This material consisted of (i) strains which have been subjected to selection and a fair degree of uniformity attained, (ii) indigenous plants from old sod in the Ottawa locality, and (iii) plants grown from commercial seed. The object of the study has been to ascertain the variability in chromosome number in plants from the various sources and to determine whether seed production is sexual or asexual.

Description of Material

Ottawa No. 1. Selection in this strain has been carried on by the Division of Forage Plants during the past 15 years. It is a very uniform upright hay type which flowers earlier than any other selection (about May 20 at Ottawa). It is a good seed producer.

Aberystwyth No. 993. A very uniform pasture type, somewhat lacking in vigor and rather slow spreading. The leaves are waxy, giving the plants a distinct light green shade. It is comparatively late flowering and a fairly good seed producer.

Aberystwyth No. 994. A vigorous, spreading, narrow-leaved, pasture type. It is comparatively late flowering and a good seed producer.

Danish No. 939. A selection from a Danish pasture obtained from Dr. O. McConkey. While the strain as a whole is quite uniform it contains a small percentage of off-types. The predominant component is a uniform, wide-leaved pasture type, possessing fair vigor. It is medium-late flowering and the seed production is good.

Swedish No. 941. A uniform spreading pasture type obtained from Dr. O. McConkey. The leaves are intermediate in width and slightly waxy. It is late flowering and a good seed producer.

Mammoth No. 959. A selection from ordinary commercial material made at the Ontario Agricultural College. It is a uniform, vigorous, wide-leaved, pasture type with a tendency to become dormant in early summer. It is distinctly late flowering and a good seed producer.

Indigenous Plot. Plants taken at random from an old permanent pasture in the vicinity of Ottawa and propagated vegetatively. It is a mixture of pasture and hay types showing a wide variation in date of flowering, leaf width and general vigor.

Commercial (Local). Variable with regard to growth forms, pasture types predominating. It has a wide variation in morphological characters.

Commercial (Foreign). Variable with regard to growth forms but hay types predominating.

Somatic Chromosome Numbers

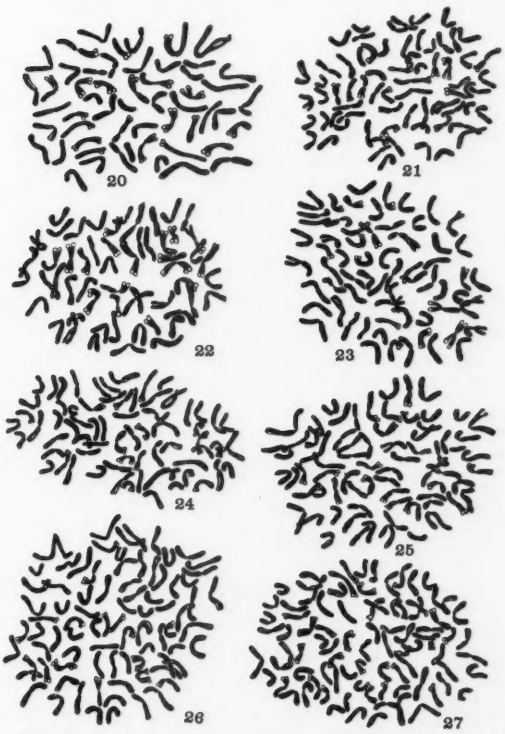
Table II gives the somatic chromosome numbers of the plants examined in the above lots. As a rule two plants were examined from each lot. In the case of Danish the plant with the lower chromosome number was an off-type. In most of the plants the chromosome number was ascertained with certainty but in certain plants, particularly the Aberystwyth, selections there may be an error of ± 1.

TABLE II

List of strains of *Poa pratensis* examined; source and somatic chromosome numbers

Source	Accession number	No. of plants examined	Chromosome numbers
Danish pasture (O.A.C.)	939	2	50, 70 ± 1
Swedish pasture (O.A.C.)	941	2	72, 72
Mammoth (O.A.C.)	959	2	54, 54
Aberystwyth	993	2	86 ± 1, 87 ± 1
Aberystwyth	994	2	84 ± 1, 84 ± 1
Ottawa selection	1	2	69 ± 1, 70
Indigenous	—	2	64, 69 ± 1
Commercial (Local)	—	2	50, 56
Commercial (Foreign)	—	3	56, 56, 65

Of the 19 plants examined, nine have euploid and ten have aneuploid chromosome numbers. All the euploids are even multiples of the basic number, 7, and are therefore capable of regular sexual reproduction. Of the six strains produced by selection, five possessed the same chromosome number in both plants examined. In the Danish strain, the plant typical of the strain had the euploid number 70 ± 1 while the off-type plant had 50. The five plants examined from commercial material showed the euploid number ($2n = 56$) for three of the plants and the aneuploid numbers of 50 and 65 for the remaining plants. The two plants from indigenous material had a euploid number for one plant and an aneuploid number for the other. Typical plates of the various plants as examined are illustrated in Figs. 20–27. They all show about the same variability in chromosome length, with median or submedian attachment constrictions. Swedish No. 941 (Fig. 6), with the aneuploid number $2n = 72$, possessed two chromosomes which are considerably shorter than the rest of complement, which suggests that they may be duplicate fragments. There is, therefore, a possibility of this biotype being derived from the euploid ($2n = 70$) by fragmentation.



FIGS. 20–27. *Biotypes of Poa pratensis L.* FIG. 20. *Mammoth No. 959, 2n = 54.* FIG. 21. *Danish No. 939, 2n = 70.* FIG. 22. *Commercial (Local), 2n = 56.* FIG. 23. *Indigenous, 2n = 64.* FIG. 24. *Ottawa No. 1, 2n = 69 ± 1.* FIG. 25. *Swedish No. 941, 2n = 72.* FIG. 26. *Aberystwyth No. 964, 2 n = 83 ± 1.* FIG. 27. *Aberystwyth No. 993, 2n = 87 ± 1.*

MICROSPOROGENESIS

In studying the reduction division in the pollen mother cells, smear preparations of the anthers were made using the method devised by McClintock (8). These smear preparations were supplemented with a few slides made by the paraffin method. Side views of the cell from heterotypic metaphase to homeotypic anaphase were examined for the occurrence of lagging univalents.

The results of this study are summarized in Table III.

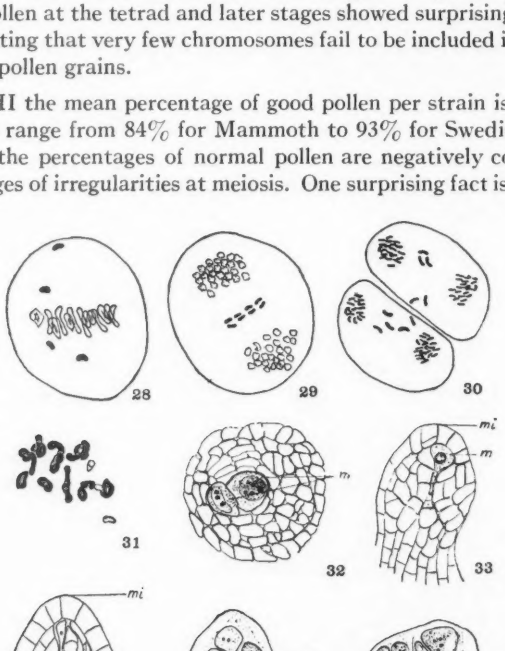
TABLE III

FREQUENCIES OF POLLEN MOTHER CELLS WITH UNIVALENT CHROMOSOMES AND PERCENTAGE OF GOOD POLLEN IN STRAINS OF *P pratensis*

——	No. of cells examined	Percentage of cells irregular	Av. no. of univalents per cell	Percentage of good pollen at dehiscence
Danish No. 839	43	2.3	.02	90.5
Swedish No. 941	25	8.0	.20	93.0
Aberystwyth No. 993	25	16.0	.28	87.0
Aberystwyth No. 994	21	32.0	.57	86.0
Mammoth No. 959	29	93.0	3.9	84.0

From the above table it may be seen that Mammoth was decidedly irregular in its meiotic behavior, the Aberystwyth strains slightly irregular, and the Danish and Swedish strains regular. Mammoth, with its very high percentage of irregular cells, afforded the best opportunity for observing the behavior of unpaired univalents. Their behavior was found to be typical of that described for the unpaired chromosomes in many interspecific hybrids (4). They divide equationally at the first division (Fig. 29), fail to divide at the second division and wander at random to either pole (Fig. 30). An examination of the pollen at the tetrad and later stages showed surprisingly few micronuclei, indicating that very few chromosomes fail to be included in the primary nuclei of the pollen grains.

In Table III the mean percentage of good pollen per strain is given. This was found to range from 84% for Mammoth to 93% for Swedish. As would be expected the percentages of normal pollen are negatively correlated with the percentages of irregularities at meiosis. One surprising fact is the relatively



FIGS. 28–31. *Meiotic divisions in Mammoth (Not all the bivalents are drawn in any given cell).* FIG. 28. *First melaphase showing four univalents off the equatorial plate.* FIG. 29. *First telophase showing univalents dividing.* FIG. 30. *Second telophase showing univalents moving at random to poles.* FIG. 31. *First metaphase in E.M.C. showing the same type of bivalent configurations as in Fig. 28; three univalents are also present.* FIG. 32. *Two sister megaspores enlarging in the initial stage of polyembryony.* FIG. 33. *Lower megaspore nearest micropyle functional.* FIG. 34. *Upper megaspore most distant from micropyle functional.* FIG. 35. *Normal embryo sac just prior to fertilization.* FIG. 36. *Polyembryony after fertilization; only one egg has been fertilized. a. antipodals; e. egg em. embryo; m, megaspore; mi, micropylar end; pn, polar nuclei; syn. synergids.*

high percentage of good pollen in the Mammoth strain. Müntzing also found the pollen quite good in the *pratensis* biotypes which he examined, even the heptaploid biotype possessing 89% good pollen, in spite of considerable irregularity at meiosis.

Pollen Tube Growth

In the study of pollen germination and pollen tube growth, panicles of the plants in which many of the florets had shed their pollen a few hours previously were fixed and stored in 15% formalin. The pistils were then dissected out and mounted in lactic acid to which a few drops of aniline blue had been added.

Germination was found to be quite active, with considerable penetration of the pollen tubes into the stigmas and style. It was obviously impossible to make a statistical comparison of pollen germination for the various strains by the above method since most of the pollen that failed to germinate floated away in the mounting fluid. Each stigma had from 30 to 100 pollen grains actively germinated. Even the Mammoth strain, which showed a high proportion of irregularities at meiosis, showed good germination of the pollen. From this study it was concluded that the plants in all the strains examined produce abundant quantities of morphologically good pollen capable of germination.

Embryology

The observations on embryology covered the period from the prophase in the E.M.C. to post-fertilization, when the embryo had reached the several-celled stage. The observations were confined to plants from the selected strains, with the omission of Ottawa No. 1. Special emphasis was laid on Mammoth which showed the highest percentage of irregularities in the P.M.C. Daily fixations of the material were made with Navashin's and Bouin's fixatives. The whole floret with enclosed anthers and pistil was sectioned longitudinally at a thickness of 15μ and stained with gentian violet.

A generalized description of the course of embryo sac development applies to all the material examined. Departures will be noted later. The megaspore mother cell undergoes a heterotypic and homeotypic division to give rise to a row of four megaspores. A critical point in this study was to determine whether actual reduction in the chromosome number takes place. Pairing of chromosomes at the heterotypic metaphase was observed for all the strains as well as disjunction at anaphase. Observations of the homeotypic division were rarer but a few cells were observed at this stage. Fig. 31 illustrates a portion of a heterotypic metaphase in Mammoth. Not all the bivalents in the plate could be drawn, but a few bivalents typical of those observed in P.M.C. reduction were apparent. Three univalents could also be distinguished.

After the formation of the row of megaspores the one destined to function as the embryo sac gradually enlarges and the remaining three disintegrate. There did not seem to be any regularity as to the position of the functional

megaspore in the row, although it was more frequently the inner one farthest from the micropyle. Anderson (1) reported the same variability as to megaspore selection. Development of the female gametophyte proceeds normally, and just prior to fertilization there is present in the embryo sac a large egg and two smaller synergids at the micropylar end, two free polar nuclei in the protoplasmic strand connecting the egg and antipodals which lie at the chalazal end of the sac (Fig. 32). The antipodals are comparatively large and densely staining, varying from three to six in number.

The material which was fixed soon after anther dehiscence was examined carefully in the hope of observing the actual act of fertilization but in all observed cases the egg was either not yet fertilized or a two- to several-celled embryo was present. A significant observation was made in the strain Aberystwyth No. 994. A good polar view of a metaphase plate was obtained in a cell of the young embryo. A chromosome count that approximated the somatic number typical of the strain could readily be made. In the light of the evidence of reduction in the E.M.C. and good pollen germination this clearly points to the act of fertilization having taken place.

Polyembryony was found to be of common occurrence, although its frequency was higher in some strains than in others. This frequency was found to be 8, 11, 35, 42 and 42% for the strains 941, 939, 909, 993 and 984. It is significant that the amount of polyembryony occurring bears some relation to the frequency of univalents in the P.M.C. at meiosis. Thus, 941, which showed little irregularity at meiosis has a low frequency of polyembryony while Mammoth, which was quite irregular in its meiotic behavior has 35% polyembryony.

As to the origin of the two megaspores in the cases of polyembryony, Anderson found it difficult to determine whether the two embryo sacs arose from two separate E.M.C. or from two megaspores in a single row of megaspores, but inclines to the view that both methods of origin obtain. At the critical stage, when the method of origin can be determined, we have frequently observed two E.M.C. lying side by side in the prophase stage. As the P.M.C. in the accompanying anthers were also at diakineses or first metaphase there could be little doubt that the cells in question were egg mother cells. However, in the majority of cases of polyembryony the enlarged cells appeared to be sister megaspores.

At later stages of development one embryo sac is usually crowded out by the other. In no case following fertilization have we observed two developing embryos, although this phenomenon has been reported by Anderson. A germination test was conducted on a seed sample of Mammoth but all the germinating seed showed single plumules, indicating that only one embryo per seed was functional.

Discussion

Müntzing accounted for the occurrence of constant aneuploid chromosome numbers in certain biotypes of *P. pratensis* by assuming apomictical seed formation. In addition to cytological constancy he found that the different

biotypes were characterized by morphological constancy and good seed production. Preliminary studies on meiosis in a heptaploid biotype showed irregularities which he considered must lead to the formation of gametes with variable chromosome numbers. If these irregular gametes were functional the cytological constancy could not be preserved. Rancken found a similar situation in a Finnish biotype, but in this case the aneuploid chromosome number was not absolutely constant since it varied from 66 to 67 $\pm$ 2 ff. The evidence presented by the above authors unquestionably points to the operation of agamosporous seed formation but the present author feels that without some direct embryological evidence, the manner of reproduction in *P. pratensis*, whether sexual or asexual, cannot be definitely ascertained.

One of the strains, Mammoth No. 959, examined in the present study is similar to those examined by Müntzing and Rancken in every respect. It is uniform morphologically and a good seed producer. The two plants examined cytologically had the aneuploid chromosome number, $2n = 54$, and meiotic studies in the P.M.C. showed an average of 3.9 univalents per cell. Nevertheless embryological studies of this strain point to the mode of reproduction for this strain as being sexual.

Pollen germination and pollen tube growth were observed in all the strains. While this does not rule out agamosporous seed production, since pollen tube growth may be a required stimulus, it does meet one of the requirements of sexual reproduction.

Reduction in the E.M.C. was also observed in all the biotypes examined. This rules out the possibility of unreduced apogamy but leaves open the possibility of reduced apogamy—that is, the egg may fuse with another reduced cell in the female gametophyte, *e.g.*, a synergid, and thus restore the diploid chromosome number. We are convinced that this is not a satisfactory explanation in the case of such a strain as Mammoth which has a low frequency of univalents in both P.M.C. and E.M.C. reduction. In such cases, if the functional megaspore did not contain the exact haploid chromosome complement, a fusion of two cells after the equational divisions in the female gametophyte could not restore the normal diploid number.

One of the significant results of the embryological studies noted was the frequent occurrence of polyembryony. The possibility of one of these embryo sacs arising aposporously from a cell of the nucellus or integument was considered. In the cases of apospory reviewed by Sharp (13) this aposporous budding out of cells from nucellar tissue takes place after the normal gametophyte has reached the eight-celled stage and results in an undifferentiated group of cells, which replaces the normal gametophyte. In the present studies the twin embryos were observed to arise simultaneously and both contained the differentiated cells, egg, synergids, polar and antipodals. Hence the double embryos present in many ovaries have in all probability a common origin.

A hypothesis will now be advanced in an attempt to explain how a strain like Mammoth which apparently is reproduced sexually, may maintain a constant aneuploid chromosome number.

In the study of microsporogenesis in Mammoth an average number of four unpaired univalents per cell was observed. At the tetrad stage and later the absence of micronuclei was also noted and this indicates that the lagging univalents have been included in the primary nuclei in the majority of cases. If the assortment of these four univalents were at random in the homeotypic division the theoretical frequency of pollen grains containing 0, 1, 2, 3, and 4 univalents (in addition to the fixed number 25) would be 1, 4, 6, 4 and 1 respectively. On this basis 6/16 of the pollen grains would contain the normal haploid number of 27 chromosomes. On this hypothesis it would be necessary to assume that only pollen grains with the normal chromosome complement function and this assumption is in agreement with results obtained in wheat species crosses (15). Considering the high chromosome number of Mammoth and other *pratensis* biotypes it is unlikely that the genetic balance would be disturbed by the random segregation of the univalents. If it were disturbed, and genetic balance as well as constant chromosome number were essential, then the production of viable pollen grains would be much reduced.

In the embryological studies a variation in the position of the functioning megaspore in the row of four megaspores was noted. This, we believe, provides a mechanism for the elimination of megaspores with an abnormal chromosome complement and for the choice of the megaspore containing the normal chromosome complement. Assuming the same frequency of irregularity in megasporogenesis as in microsporogenesis, 6/16 of the megaspores would be normal, and with a choice of four megaspores the chance of obtaining one with the normal number is quite good. As previously shown the frequency of polyembryony in the different strains is related to the degree of irregularity of meiosis. Strains 939 and 941, which are almost completely regular at meiosis, have a low frequency while Mammoth has a high frequency of polyembryony associated with its irregular meiotic behavior.

The phenomenon of polyembryony, both in the development of two megaspores and the later elimination of the additional embryo sac, may be regarded as evidence that there is a selective tendency in certain strains of *P. pratensis* towards a choice of normal megaspores. Polyembryony appears to be a response to meet the situation of irregular chromosome behavior.

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THE PHYSIOLOGY OF THE SHEEP TAPEWORM, *MONIEZIA EXPANSA* BLANCHARD[1]

BY ROBERT ARNOLD WARDLE[2]

Abstract

The influence of saline media upon the longevity, water content and polysaccharide content of *Moniezia expansa* is discussed. Worms may live two or three days in balanced salines but in the majority of experiments they die between 9 and 12 hours. Addition of glucose abbreviates the longevity period in saline media. The water content is affected only slightly by balanced salines but is influenced by the concentration of sodium chloride and by the presence of sugars and amino acids; behavior in saline media cannot be correlated wholly with changes in water content. The polysaccharide content is not significantly different from mammalian glycogen, constitutes 0.35 to 5.25% of the fresh weight, remains stable during immersion of the living worm for six hours in media that reduce muscle tonus, but decreases during immersion in media that encourage muscle tonus. Glycogen increase occurs when the medium contains glucose up to 1%, but not when the glucose content exceeds this amount or when the glucose is replaced by other sugars, by amino acids, or by glycoproteins. Saline media are adjudged unfavorable to tapeworm existence *in vitro*.

1. Introduction

The literature on the subject of tapeworm physiology is scanty and the neglect of this aspect of comparative physiology can only be attributed to the lack of established technique for keeping a tapeworm alive outside its host for a sufficient length of time to permit of physiological experimentation and observation. So closely adapted is an adult tapeworm to its habitat—which, with few exceptions, is the small intestine of some chordate animal—that its life in the relatively crude laboratory imitations of this habitat is always brief, the animal disintegrating with rapidity whether in sterile or in oligoseptic media.

The data presented below are the results of a series of experiments extending over three years with a typical tapeworm *Moniezia expansa*, employing the saline nutrient media commonly used for animal cultivation experiments, and taking as criteria of the favorableness of such media their influence upon the longevity of the tapeworm, upon its water content, and upon its polysaccharide content.

2. Material and Methods

The sheep tapeworm, *Moniezia expansa* (Rudolphi, 1810) Blanchard, 1891, was selected as a subject for this study because it is abundant and easy to obtain during the late summer in Canada, and because it has been used by

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several other workers in this field, notably by Brand, and Cook and Sharman, the latter workers referring to their material erroneously as "*Moniezia trisonophora*". It is, however, not an ideal tapeworm for experiments of this nature since it cannot be obtained in quantity during the winter and spring, and is somewhat sensitive to removal from the host.

The tapeworms can be obtained most readily and quickly by hand-squeezing the lamb gut slowly from the pylorus to the beginning of the large intestine, and although each lamb may contain only 4–10 worms, the large scale on which lambs are slaughtered in the Canadian packing industry, and the high incidence of this worm in western Canadian lambs, permit the collection of several hundred worms per hour. Medium sized specimens, 30–50 cm. in length, that show activity and are unstained by bile, should be selected, rinsed in physiological saline, drained on soft cotton cloth, and placed as soon as possible in the experimental media. If kept at 20° C. in moist cotton cloth, however, they will live with negligible muscular activity and negligible polysaccharide and water loss up to 12 hours.

The media used were unsterilized but freshly prepared saline solutions alone or plus the addition of 1% of some nutrient substance,—sugars, amino acids, glycoproteins. Four saline bases were used in most of the experiments:

1. *Tyrodes solution*, used in single strength and two and a half times single strength; the stronger solution was used on the assumption, that later proved erroneous, that it would be nearer isotonicity for the worms than would the ordinary concentration.

2. *Phosphate saline*, which was Tyrodes solution with the sodium acid phosphate content increased from 0.02 to 0.2%, on the suggestion of Magee and Reid (10) that the presence of 0.2% of sodium acid phosphate stimulates the absorption of glucose by the intestinal mucosa of the rat.

3. *Bile saline*, a solution of 0.8% sodium chloride in water plus the addition of 10% fresh sheep bile, this concentration of bile being assumed to resemble the concentration present in the small intestine of the lamb.

4. *Sodium glycocholate*, in 1% aqueous solution, used as a ready solvent for certain nutrients (lecithin, cholesterol) not soluble in the other bases.

In the water content experiments, tests were made also with distilled water, half, double, triple, quadruple and quintuple strength Tyrodes solution; with a Ringer solution formula recommended by Brand (4); and with another Ringer solution formula recommended by Cook and Sharman (6); and with $M/5$ and $M/10$ sodium chloride solutions. These media were used in 200-ml. quantities in 250-ml. Erlenmeyer type Pyrex flasks kept in a dry incubator at 36-38° C. for a period of six hours only; five worms were used in each test and the test was repeated at least three times.

It may be noted that, throughout this paper, the term *fresh weight* means the weight of freshly collected, living material, after rinsing in physiological saline and draining on soft cotton cloth until the surface is barely moist;

the term *alcoholic weight* means the weight of tapeworm material after storage for one month in 95% ethanol, and subsequent drainage until superficially dry; and *dry weight* means the weight of the worm material after desiccation to constant weight, usually for 4–5 hr. at 150° C., care being taken to avoid charring. Among 100 specimens, the alcoholic weight was found to be 50.1% of the fresh weight, the dry weight was 10.1% of the fresh weight.

3. Tapeworm Longevity

Very little accurate information is available concerning the longevity of tapeworms whether *in situ* or *in vitro*. The author (18) has summarized previously such information as was available up to 1933, and can add to that summary merely the observation by Seddon (13) that in Australian lambs *Moniezia expansa* lives not longer than 65–70 days; the observation by Gordon (8) that in Australian lambs *Moniezia expansa* produces eggs for at least 350 days; and the observations of Leiper (9) of the persistence of *Diphyllobothrium latum* in man during five years and of *Diphyllobothrium mansoni* in dogs for eight years.

The difficulty in observations of tapeworm longevity *in situ* is that of eliminating the possibility of host re-infestation, a difficulty that has been stressed by Ward (16) in a critical analysis of a large number of cases of reputed longevity in *Diphyllobothrium latum*.

The difficulty in observations of tapeworm longevity *in vitro* is that of deciding whether the tapeworms are alive at all, and of deciding the exact moment of death. Absolute values of tapeworm longevity *in vitro* are of little value as physiological data since for part of the time the animal may be moribund or disintegrating. Nor can it be assumed, as was done by Cook and Sharman (6) that carbon dioxide production is indicative of tapeworm vitality, since the experiments of Brand and Weise (5) suggest that carbon dioxide production by tapeworms is a by-product of glycogenolysis and not a by-product of aerobic respiration, and it is possible that such glycogenolysis is as much a post-mortem as a pre-mortem phenomenon. In the light of Brand's results, the belief of Cook and Sharman that *Moniezia expansa* will live 200 hours in their saline solution plus $M/10{,}000$ sodium hydroxide needs confirmation.

The author (17, 18) has attempted to show in previous papers that tapeworms *in vitro* pass through a period of undulant activity, sometimes brief, sometimes prolonged, before passing eventually into either a condition of flaccidity or a condition of tetanic contraction, both irreversible, and has argued that the termination of longevity should be taken arbitrarily as the moment when undulant activity ceases.

A series of experiments was carried out to determine the duration of undulant activity of *Moniezia expansa* in Tyrode's solution, Brand's solution, Cook and Sharman's solution, $M/5$ sodium chloride and $M/10$ sodium chloride. These salines were used alone, and again with glucose added in amounts

equivalent to $M/5$, $M/10$, $M/100$, $M/500$ and $M/1000$. One set of experiments was made with sterilized media, one set with unsterilized but freshly prepared media. Each experiment was repeated five times, five worms being used each time under the conditions of temperature given above. Observations were made at hourly intervals.

The results obtained were extremely unsatisfactory. The moment of transition from activity to passivity is difficult to determine among a number of intertwined worms and there is considerable variation between individuals. The general findings were as follows:—

1. In glucose-free media or media with $M/500$ or less of glucose, the activity period varied from 24–72 hr. in Brand's solution, 24–48 hr. in Tyrode's solution, 9–12 hr. in Cook and Sharman's solution, 9–12 hr. in $M/5$ and $M/10$ sodium chloride.

2. In media containing glucose exceeding $M/500$ in amount, the period of undulant activity was always short and varied from 9–12 hr.

In none of the media that were tested, whether sterile or non-sterile, whether glucose-free or glucose-containing, at hydrogen ion concentrations between 6.0 and 9.0, could specimens of *Moniezia expansa*—only one hour distant from life *in situ*—be relied upon to remain undulant longer than 12 hr. although occasional specimens might remain so for two or three days. The fact seems to be that no two individuals of a tapeworm species will react in the same way to the adverse conditions of an artificial environment.

Taking undulant activity as a criterion of the favorableness of an artificial medium to *Moniezia expansa*, fluid saline media are unfavorable to its life *in vitro*.

No reason for the brevity of the undulant activity period in saline media can be given. The duration of the experiment was too short for such inhibition to be caused by bacterial toxins or by the by-products of metabolism–carbon dioxide, succinic acid, lactic acid. Stoppage of activity in a sugar-free medium might be suspected to arise from glycogen exhaustion were it not for the fact that, as shown in later experiments, the glycogen content is not rapidly exhausted by starvation, and in fact is considerably increased by the presence of glucose in the medium.

4. Water Content

The author (17, 18) has discussed in previous papers the change of form induced in living tapeworms by immersion in media that are hypotonic and hypertonic in the physiological sense; how tapeworms tend to expand and become flaccid in hypotonic salines, and to become increasingly foreshortened and contracted in media whose electrolyte content is gradually increased from isotonicity.

That such morphic changes are associated with changes in water content might seem to be suggested by the high water content of tapeworms (usually between 85 and 90%), by the absence from tapeworms of exoskeletal and endoskeletal structures, and by the admitted porosity of the tapeworm surface.

Experimental confirmation of this suggestion is illustrated by Table I, which indicates the mass per original gram of living *Moniezia expansa* after immersion for six hours at 36–38° C. in various aqueous media.

TABLE I

MASS PER ORIGINAL GRAM OF LIVING *Moniezia expansa* AFTER SIX HOURS' IMMERSION AT 36–38° C.

		Arabinose	Glucose	Galactose	Levulose	Maltose	Glycine	Mucin
Water	3.3							
Tyrode × ½	2.0							
Tyrode × 1	1.004	1.16	1.15	1.04	1.04	1.15	1.87	1.28
Tyrode × 2	0.70							
Tyrode × 2½	0.88	0.53	0.71	0.38	0.67	0.50	0.52	0.56
Tyrode × 3	0.88							
Tyrode × 4	0.76							
Tyrode × 5	0.61							
Sod. glycol	0.83	0.88	0.96	0.86	0.84	0.91	0.67	—
Bile saline	1.01	0.82	0.98	0.60	0.86	0.82	0.74	1.33

	Glucose, %							
	—	0.1	0.5	1.0	2.0	5.0	10.0	20.0
Phosphate saline	0.99	1.09	0.94	0.82	0.50	0.38	0.38	

Selected whole worms were used. They were rinsed, drained on soft cotton cloth, weighed, immersed in the medium for six hours, then removed, rinsed, drained, and weighed again. The media used are named in the table.

Any change in weight induced in a tapeworm by brief immersion in a fluid must be due to water loss or water gain, since the glycogen or fat changes during six hours represent extremely minute fractions of the worm's weight. The data presented in Table I suggest that least disturbance of the initial water content is induced by Tyrode's solution, phosphate saline, and bile saline, that is to say, by those media that are isotonic with mammalian tissues. In hypotonic media such as water and half-Tyrode, there is absorption of water which may account to some extent for the flaccidity of tapeworms in such solutions. As the sodium chloride content of the medium is increased, the tapeworm shows increasing water loss until, when concentrations greater than quadruple strength are used, the tapeworm is definitely losing water and contracting visibly.

The influence of added nutrients upon water content seems to vary according to the saline base, but glucose in amounts greater than 1% seems to induce water loss.

The relatively slight disturbance of the tapeworm water content by isotonic saline media would suggest that on this criterion such media are suitable for tapeworm cultivation *in vitro*. Since, however, in such media the tapeworm is far more active and more extended than it is when *in situ*, muscular activity must to some extent be influenced by penetration of ions or molecules from the medium, a conclusion arrived at previously by the author (18), from experiments with *Nybelinia surmenicola*.

5. Polysaccharide Content

Appreciable quantities of a polysaccharide substance have been shown to occur in such gut-frequenting helminths as have been analyzed, and in the germinal membrane of the vesicular larvae of taeniid tapeworms. Reference should be made to Brand (3, 4, 5) and to Schopfer (12) for a résumé of the existing literature on the question.

Among 30 specimens of *Moniezia expansa* analyzed by Brand (4) the polysaccharide content, expressed in terms of reducing sugar to bring the figures into line with those presented below, and assuming that Brand used Pflüger's factor of 0.927, ranged from 0.99 to 5.35%, with a mean value of 2.69% of the fresh weight. This polysaccharide content may be isolated by the method of Good, Kramer and Somogyi (7) and its reducing sugar content estimated iodometrically by the method of Shaffer and Hartmann (14); or it may be further purified by the method of Somogyi (15) and dried over calcium chloride. Thus prepared from *Moniezia expansa*, the polysaccharide constituent is an amorphous white powder, readily soluble in water, beginning to char at 210° C. and attaining maximum charring at 245° C. When hydrolyzed by heating for three hours in 3% hydrochloric acid at 90° C., it yields a reducing sugar content of 95.27%; the nature of the residue was not determined. The reducing sugar obtained answers all the recognized criteria of *d*-glucose, and the polysaccharide appears therefore not significantly different from mammalian glycogen.

Among 16 selected worms, analyzed within one hour of removal from the host, the polysaccharide content expressed in terms of reducing sugar, and using the conversion factor of 0.961 suggested by Bell and Young (1), ranged from 0.35 to 5.23% of the fresh weight, with a mean value of 3.14%. Among 17 worms analyzed after one month of storage in 95% ethyl alcohol, the polysaccharide content similarly expressed ranged from 0.52 to 4.89% of the fresh weight, with a mean value of 3.31%. Among 400 thirds of *Moniezia* individuals, after one month of storage in ethyl alcohol, the polysaccharide content ranged from 1.55 to 3.96% of the fresh weight, with a mean value of 3.22%. Ethyl alcohol therefore, as could be expected, inhibits glycogenolysis and is a convenient storage fluid for material whose polysaccharide content is to be estimated. On the other hand, among 10 specimens stored for one month in 10% formalin, the polysaccharide content was only 0.19% of the fresh weight, so that formalin-preserved tapeworms are useless for glycogen determination.

The polysaccharide content of *Moniezia expansa*, therefore, is relatively high, since it constitutes approximately 3.2% of the fresh weight, or approximately 32% of the dry weight and may exceed the fat content or the protein content. Microscopical examination of sectioned *Moniezia* material stained by the glycogen demonstration method of Best (2) shows the polysaccharide content to be distributed uniformly throughout the parenchyma but to be absent from the cuticle, dermis, longitudinal and circular muscles, uterine reticulum and eggs. Its absence from the muscles of worms killed by plunging

into boiling ethyl alcohol, suggests a definite correlation between polysaccharide content and muscular activity.

It is generally conceded by students of helminth physiology that the polysaccharide constituent of a parasitic helminth is synthesized from glucose withdrawn from the host fluids, similar synthesis from amino-acids being precluded by the lack of aerobic respiration, and that in the case of tapeworms such host glucose must enter through the cuticle and be synthesized into glycogen in the parenchymal cells. Ortner-Schönbach (11) suggests that the glycogen-gorged parenchyma acts as a source of glucose for the muscles, dermis and gonad-ducts where it is degraded, and as a source of sugar for the ovaries, vitellaria and gland cells where it is re-synthesized into glycogen. Admission must be made, however, that the hypothesis of glycogenesis from environmental glucose has received little experimental support. Ortner-Schönbach could detect no histological evidence of alterations in glycogen content between control specimens of the horse tapeworm *Anoplocephala* and specimens that had been kept for seven days in saline solutions and in saline-glucose solutions. Apart from the possibility that the experimental worms may not have been alive during the whole of the seven days, the present histological methods of demonstrating tissue glycogen are far from being sufficiently precise to indicate differences in glycogen concentration.

Brand (4) obtained inconclusive results by the anaerobic immersion of specimens of *Moniezia expansa* in Ringer-glucose solution. In four experiments, one worm showed a glycogen gain of 0.13% after six hours' immersion at 37.5° C. but the other three showed glycogen losses of 0.02, 0.03, and 0.18%. In the opinion of the present writer the experimental error in experiments of this nature may be as high as 1%, that is to say, two halves of the one worm may differ in glycogen content to that extent, so that Brand's results merely suggest that *no* glycogen change occurs when *Moniezia expansa* is immersed for six hours at 37.5° C. in an anaerobic solution of Ringer-glucose.

More suggestive are Brand's observations that eight specimens of *Taenia hydatigena*, from a dog that had received a high carbohydrate diet, had a glycogen content of 8.53% of the fresh weight, whereas specimens from a dog fed on an ordinary diet showed a percentage of 4.99%, but even these figures cannot be seriously considered unless it be shown that the difference, 3.39%, is greater than the maximum variation that occurs normally in glycogen content between individuals of this tapeworm species.

The data presented in Table II are based upon six-hour immersion experiments with *Moniezia expansa* in various media at temperatures of 36–38° C. In such experiments advantage may be taken of the fact that fractionated tapeworms live just as long as intact ones. Each experiment made use of five selected living worms; each was cut into approximate thirds; one set was fixed in boiling ethyl alcohol and stored in ethyl alcohol for one month before being analyzed for initial glycogen content; a second set of thirds was im-

TABLE II

INFLUENCE UPON GLYCOGEN (G) CONTENT, EXPRESSED IN MILLIGRAMS OF REDUCING SUGAR PER GRAM OF FRESH WEIGHT, OF IMMERSION OF LIVING *Moniezia* FOR SIX HOURS AT 36–38° C. IN VARIOUS MEDIA

Medium	Initial G	Final G	Medium	Initial G	Final G
Dist. water	27.32	30.42	Tyrode × 1, plus		
Tyrode × ½	29.2	28.4	Arabinose	29.8	15.3
Tyrode × 1	23.56	22.16	Glucose	30.4	55.3
Tyrode × 2	30.1	30.4	Galactose	30.0	19.0
Tyrode × 2½	35.5	36.1	Levulose	37.6	16.2
Tyrode × 3	25.84	21.25	Maltose	32.7	14.3
Tyrode × 4	31.42	25.13	Glucosamine	20.9	21.4
Tyrode × 5	38.8	20.18	Glycine	26.7	22.5
Sod. glycol.	29.7	14.7	Tyrosine	31.6	38.14
Bile saline	34.4	19.4	Mucin	40.5	18.9
Phosph. sal.	33.2	32.3	Bile 10%	20.0	17.8
Tyrode × 2½ plus			Bile saline, plus		
Arabinose	20.07	10.15	Arabinose	28.86	5.8
Glucose	31.6	39.87	Glucose	26.62	41.4
Galactose	33.5	27.5	Galactose	25.08	14.29
Levulose	29.12	27.72	Levulose	38.8	14.08
Maltose	37.00	22.5	Maltose	31.07	7.09
Glycine	30.3	11.3	Nutrient agar	30.2	5.6
Glucosamine	36.4	34.8	Glucosamine	47.16	35.7
Mucin	24.3	25.1	Mucin	36.6	37.8
Sodium glycocholate, plus			Phosphate saline plus		
Arabinose	34.8	31.0	Glucose, 0.1%	39.3	28.6
Glucose	32.68	37.48	Glucose, 0.5	21.4	52.1
Galactose	21.9	21.6	Glucose, 1.0	41.2	27.0
Levulose	36.8	11.7	Glucose, 2.0	31.5	0.93
Maltose	39.8	13.08	Glucose, 5.0	32.05	1.40
Glycine	38.36	33.8	Glucose, 10.0	41.6	6.8
Tyrosine	38.34	31.2	Glucose, 20.0	41.2	7.8
Mucin	28.06	26.0			
Cholesterol	36.06	4.06			
Lecithin	24.2	4.07			

mersed in the saline base for six hours, then alcoholized and stored; the remaining set of thirds was immersed for six hours in the saline base plus the nutrient, and then alcoholized and stored. Each experiment was repeated three times.

In the table the polysaccharide contents are expressed in terms of milligrams of reducing sugar per gram of fresh weight, allowance being made for water content changes induced by the media.

The differences between pre-experimental and post-experimental polysaccharide contents are of significance only where they exceed the maximum variation that may occur in polysaccharide content between successive thirds of a group of five worms. To arrive at this, four groups of five trisected worms were taken and each set of thirds analyzed after one month in alcohol storage. For each of the four groups, the maximum differences in polysaccharide content between sets of thirds were 2.0, 4.2, 6.0 and 10.0 milligrams of reducing sugar per gram of fresh weight.

In the table, therefore, differences of less than 10 mg. between pre-experimental and post-experimental data must be discarded as without significance.

It may be assumed, from the data in Table I, that when living specimens of *Moniezia expansa* are immersed in saline media for six hours at 36–38° C., under aerobic conditions that:

(1) There are no significant changes in polysaccharide content induced by immersion in distilled water, half Tyrode, full Tyrode, double, two and a half, triple, quadruple Tyrode, and in phosphate saline; that is to say, in media in which the longitudinal musculature of the tapeworm, as compared with the longitudinal musculature of the worm *in situ*, is relaxed;

(2) There is polysaccharide loss induced by immersion in quintuple Tyrode, in 1% sodium glycocholate solution, and in bile saline; that is to say, in those media in which contraction of the worm occurs or where it vigorously undulates. Tempting as it is to assume that the polysaccharide loss represents consumption by the functioning musculature, it is not impossible that despite its large molecule, glycogen may pass out through the body surface with the abstracted water. Glycogen can be detected in the mucus covering the freshly collected worms in quantity as high as 1.3 mg. per gm. fresh weight. The loss of glycogen in bile saline does not fit in with either conception since the water content loss in bile saline is negligible, and the undulant activity is scarcely more vigorous than when the worm is in Tyrode solution;

(3) There is a gain in polysaccharide content when glucose is present in concentrations of 1% in Tyrode's solution, bile saline, and probably two and a half Tyrode, but in sodium glycocholate and phosphate saline the polysaccharide content in the presence of 1% glucose remains unchanged;

(4) No polysaccharide gain was obtained by the substitution for glucose of other sugars, of amino acids and of glycoproteins, nor, in phosphate saline, of higher concentrations of glucose.

The glycogen changes shown above do not altogether support the view of Ortner-Schönbach (11) that the primary function of tapeworm glycogen is the nourishment of the developing genitalia, but suggest rather that it is primarily a source of fuel for the longitudinal musculature when the latter is maintaining the condition of muscular tonus which characterizes the normal worm, and that it is depleted in any artificial medium in which this tonus is maintained or in which it is exaggerated into tetanic contraction, and depleted only very slowly or scarcely at all when the musculature relaxes completely or undergoes periodically repeated phases of relaxation as during undulant activity.

6. Conclusions

If maintenance of normal longevity, water content and polysaccharide content be accepted as criteria of the favorableness of artificial media to tapeworms, and if *Moniezia* be regarded as representative of tapeworms in general, the inescapable conclusion from the data presented above is that the saline media usually employed in physiological studies are useless for the study of tapeworm physiology, and that conclusions as to the nature of tapeworm metabolism that are based upon experiments carried out in such media cannot be accepted as giving an accurate picture of the physiological processes taking

place in the tapeworm that is lying in an animal gut. That is to say, the bulk of information already accumulated upon tapeworm physiology, scanty as it is, is practically worthless, and workers in this field of physiology will have to adopt a technique more akin to that of the bacteriologist than to that of the physiologist. Successful tapeworm cultivation *in vitro* may require that the animal be embedded in a semi-fluid gel after preliminary aseptic treatment. The technique of such asepsis has yet to be established, and the author is unaware of any experiments in this direction beyond his own crude and unsuccessful experiments with *Nybelinia surmenicola* (18). On the other hand, the nutritional requirements of such a tapeworm when *in vitro* may prove to be less exacting than has been supposed and it may not be necessary to duplicate the nutrient complex that surrounds the tapeworm *in situ*. Glucose seems definitely the one carbohydrate requirement of tapeworms; the amino acid requirement may be equally simple; there may be no fat requirement.

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